



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 16/24, C12N 15/13, 15/63, 5/10, C07K 16/00, A61K 39/395, G01N 33/577, C12P 21/08, A61P 43/00	A1	(11) International Publication Number: WO 00/56772 (43) International Publication Date: 28 September 2000 (28.09.00)
(21) International Application Number: PCT/US00/07946 (22) International Filing Date: 24 March 2000 (24.03.00) (30) Priority Data: 60/126,603 25 March 1999 (25.03.99) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/126,603 (CIP) Filed on 25 March 1999 (25.03.99) (71) Applicants (for all designated States except US): BASF AK-TIENGESELLSCHAFT [DE/DE]; Carl-Bosch Strasse 38, D-67056 Ludwigshafen (DE). GENETICS INSTITUTE INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SALFELD, Jochen, G. [DE/US]; 177 Old Westborough Road, North Grafton, MA 01536 (US). ROGUSKA, Michael [US/US]; 16 Hilldale Road, Ashland, MA 01721 (US). PASKIND, Michael [US/US]; 253 Redemption Rock Trail, Sterling,		MA 01564 (US). BANERJEE, Subhashis [IN/US]; 25 Hapgood Way, Shrewsbury, MA 01545 (US). TRACEY, Daniel, E. [US/US]; 149 Shaker Road, Harvard, MA 01451 (US). WHITE, Michael [US/US]; 30 Angelica Drive, Framingham, MA 01701 (US). KAYMAKALAN, Zehra [TR/US]; 4 Picadilly Way, Westborough, MA 01581 (US). LABKOVSKY, Boris [US/US]; 107A-5 Broadmeadow Road, Marlborough, MA 01752 (US). SAKORAFAS, Paul [US/US]; 47 Court Street, Newton, MA 02160 (US). FRIEDRICH, Stuart [CA/US]; 20 Albion Street, Melrose, MA 02176 (US). MYLES, Angela [US/US]; Apartment #7, 11 Crescent Drive, Andover, MA 01810 (US). VELDMAN, Geertruida, M. [NL/US]; 60 Woodmere Drive, Sudbury, MA 01776 (US). VENTURINI, Amy [US/US]; 207 Woburn Street, Lexington, MA 02420 (US). WARNE, Nicholas, W. [US/US]; 27 Farrwood Drive, Andover, MA 01810 (US). WIDOM, Angela [US/US]; 19 Cherokee Road, Acton, MA 01720 (US). ELVIN, John, G. [GB/GB]; 36 Perowne Street, Cambridge CB1 2AY (GB). DUNCAN, Alexander, R. [GB/GB]; 3 Hauwxton Road, Little Shelford, Cambridge CB2 5HJ (GB). DERBYSHIRE, Elaine, J. [GB/GB]; 15 The Brambles, Studlands Rise, Royston, Hertfordshire SG8 9NQ (GB). CARMEN, Sara [GB/GB]; 136 Sturton Street, Cambridge CB1 2QF (GB). SMITH, Stephen [GB/GB]; 33 Church Road, Wicken, Ely, Cambridgeshire CB7 5XT (GB). HOLTET, Thor, Las [DK/GB]; 13 Mortimer Road, Royston, Hertfordshire (GB). DU FOU, Sarah, L. [GB/GB]; 76 Ickleford Road, Hitchin, Hertfordshire SG5 1TL (GB). (74) Agents: KARA, Catherine, J. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US). (81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING		
(57) Abstract Human antibodies, preferably recombinant human antibodies, that specifically bind to human interleukin-12 (hIL-12) are disclosed. Preferred antibodies have high affinity for hIL-12 and neutralize hIL-12 activity <i>in vitro</i> and <i>in vivo</i> . An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. The antibodies, or antibody portions, of the invention are useful for detecting hIL-12 and for inhibiting hIL-12 activity, e.g., in a human subject suffering from a disorder in which hIL-12 activity is detrimental. Nucleic acids, vectors and host cells for expressing the recombinant human antibodies of the invention, and methods of synthesizing the recombinant human antibodies, are also encompassed by the invention.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING

Related Applications

5 This application is a non-provisional application claiming priority to U.S. provisional application Serial No. 60/126,603, filed March 25, 1999, the contents of which are hereby incorporated by reference.

Background of the Invention

10 Human interleukin 12 (IL-12) has recently been characterized as a cytokine with a unique structure and pleiotropic effects (Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237). IL-12 plays a critical role in the pathology associated with several diseases involving
15 immune and inflammatory responses. A review of IL-12, its biological activities, and its role in disease can be found in Gately *et al.* (1998) *Ann. Rev. Immunol.* 16: 495-521.

 Structurally, IL-12 is a heterodimeric protein comprising a 35 kDa subunit (p35) and a 40 kDa subunit (p40) which are both linked together by a disulfide bridge (referred to as the "p70 subunit"). The heterodimeric protein is produced primarily by antigen-
20 presenting cells such as monocytes, macrophages and dendritic cells. These cell types also secrete an excess of the p40 subunit relative to p70 subunit. The p40 and p35 subunits are genetically unrelated and neither has been reported to possess biological activity, although the p40 homodimer may function as an IL-12 antagonist.

 Functionally, IL-12 plays a central role in regulating the balance between antigen
25 specific T helper type (Th1) and type 2 (Th2) lymphocytes. The Th1 and Th2 cells govern the initiation and progression of autoimmune disorders, and IL-12 is critical in the regulation of Th₁-lymphocyte differentiation and maturation. Cytokines released by the Th1 cells are inflammatory and include interferon γ (IFN γ), IL-2 and lymphotoxin (LT). Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 to facilitate humoral immunity,
30 allergic reactions, and immunosuppression.

Consistent with the preponderance of Th1 responses in autoimmune diseases and the proinflammatory activities of IFN γ , IL-12 may play a major role in the pathology associated with many autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and Crohn's disease.

5 Human patients with MS have demonstrated an increase in IL-12 expression as documented by p40 mRNA levels in acute MS plaques. (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996). In addition, *ex vivo* stimulation of antigen-presenting cells with CD40L-expressing T cells from MS patients resulted in increased IL-12 production compared with control T cells, consistent with the observation that CD40/CD40L
10 interactions are potent inducers of IL-12.

Elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita *et al* (1998) *Arthritis and Rheumatism*. 41: 306-314). Cytokine messenger ribonucleic acid (mRNA) expression profile in the RA synovia identified predominantly Th1 cytokines. (Bucht *et al.*, (1996) *Clin. Exp.*
15 *Immunol.* 103: 347-367). IL-12 also appears to play a critical role in the pathology associated with Crohn's disease (CD). Increased expression of IFN γ and IL-12 has been observed in the intestinal mucosa of patients with this disease (Fais *et al.* (1994) *J. Interferon Res.* 14:235-238; Parronchi *et al.*, (1997) *Am. J. Path.* 150:823-832; Monteleone *et al.*, (1997) *Gastroenterology*. 112:1169-1178, and Berrebi *et al.*, (1998)
20 *Am. J. Path* 152:667-672). The cytokine secretion profile of T cells from the lamina propria of CD patients is characteristic of a predominantly Th1 response, including greatly elevated IFN γ levels (Fuss, *et al.*, (1996) *J. Immunol.* 157:1261-1270). Moreover, colon tissue sections from CD patients show an abundance of IL-12 expressing macrophages and IFN γ expressing T cells (Parronchi *et al* (1997) *Am. J.*
25 *Path.* 150:823-832).

Due to the role of human IL-12 in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract IL-12 activity. In particular, antibodies that bind to, and neutralize, IL-12 have been sought as a means to inhibit IL-12 activity. Some of the earliest antibodies were murine monoclonal antibodies (mAbs),
30 secreted by hybridomas prepared from lymphocytes of mice immunized with IL-12 (see *e.g.*, World Patent Application Publication No. WO 97/15327 by Strober *et al.*; Neurath *et al.* (1995) *J. Exp. Med.* 182:1281-1290; Duchmann *et al.* (1996) *J. Immunol.* 26:934-

- 3 -

938). These murine IL-12 antibodies are limited for their use *in vivo* due to problems associated with administration of mouse antibodies to humans, such as short serum half life, an inability to trigger certain human effector functions and elicitation of an unwanted immune response against the mouse antibody in a human (the "human anti-
5 mouse antibody" (HAMA) reaction).

In general, attempts to overcome the problems associated with use of fully-murine antibodies in humans, have involved genetically engineering the antibodies to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains
10 are human-derived, have been prepared (Junghans, *et al.* (1990) *Cancer Res.* 50:1495-1502; Brown *et al.* (1991) *Proc. Natl. Acad. Sci.* 88:2663-2667; Kettleborough *et al.* (1991) *Protein Engineering*, 4:773-783). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction,
15 especially when administered for prolonged periods.

A preferred IL-12 inhibitory agent to murine antibodies or derivatives thereof (*e.g.*, chimeric or humanized antibodies) would be an entirely human anti-IL-12 antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods. However, such antibodies have not been described in the art and,
20 therefore are still needed.

Summary of the Invention

The present invention provides human antibodies that bind human IL-12. The invention also relates to the treatment or prevention of acute or chronic diseases or
25 conditions whose pathology involves IL-12, using the human anti-IL-12 antibodies of the invention.

In one aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that binds to human IL-12.

In one embodiment, the invention provides a selectively mutated human IL-12
30 antibody, comprising:

- 4 -

a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue such that it binds to human IL-12.

In a preferred embodiment, the invention provides a selectively mutated human
5 IL-12 antibody, comprising:

a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position with an activity enhancing amino acid residue such that it binds to human IL-12.

In another preferred embodiment, the selectively mutated human IL-12 antibody
10 or antigen-binding portion thereof is selectively mutated at more than one preferred selective mutagenesis position, contact or hypermutation positions with an activity enhancing amino acid residue. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than three preferred selective mutagenesis positions, contact or hypermutation
15 positions. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than two preferred selective mutagenesis position, contact or hypermutation positions. In yet another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof, is selectively mutated such that a target specificity
20 affinity level is attained, the target level being improved over that attainable when selecting for an antibody against the same antigen using phage display technology. In another preferred embodiment, the selectively mutated human IL-12 antibody further retains at least one desirable property or characteristic, *e.g.*, preservation of non-cross reactivity with other proteins or human tissues, preservation of epitope recognition,
25 production of an antibody with a close to a germline immunoglobulin sequence.

In another embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a K_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro*
30 phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less. More preferably, the isolated human antibody or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-2} \text{ s}^{-1}$ or less, or

inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-7} M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-3} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-8} M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-4} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-10} M or less. Even more preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-11} M or less.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-6} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6. In a preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

- 6 -

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 5 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the isolated human antibody, or an antigen-binding
10 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID
15 NO: 14. In a preferred embodiment, the isolated human antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 20 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID
25 NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20. In a preferred embodiment, the isolated human antibody, or an antigen-binding
30 portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22. In a preferred embodiment, the isolated human antibody, or an antigen-binding

- 7 -

portion thereof, has the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions or any allelic variation thereof as discussed in Kabat *et al.* (Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), included herein by reference. In a more preferred embodiment, the antibody heavy chain constant region is IgG1. In another preferred embodiment, the isolated human antibody is a Fab fragment, or a F(ab')₂ fragment or a single chain Fv fragment.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant

- 8 -

region, or an Fab fragment or a F(ab')₂ fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID
10 NO: 26.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28. In a preferred embodiment, the isolated human antibody, or an antigen-binding
15 portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino
20 acid sequence of SEQ ID NO: 32. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region, or an Fab fragment, or a F(ab')₂ fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 25 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁶ M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5,
30 or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid

- 9 -

sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and

- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain

- 10 -

CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;

 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain
10 CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and

 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.
15

25 The invention also provides nucleic acid molecules encoding antibodies, or antigen binding portions thereof, of the invention. A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17. The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 19. In another
30 embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 21. In another

embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18. The isolated nucleic acid encoding an antibody light chain variable
5 region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 20. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 22. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable
10 region comprising the amino acid sequence of SEQ ID NO: 24.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 15 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein
20 said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of
25 SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein
30 said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25. The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 27. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29. In another embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. The isolated nucleic acid encoding an antibody light chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 28. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 30. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

In another aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the $V_{\text{H}}3$ germline family, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from a member of the $V_{\lambda}1$ germline family, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact position or hypermutation position with an activity enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
5 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact
10 position or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

15 In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
20 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

25 c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an
30 antigen-binding portion thereof, which has the following characteristics:

- 14 -

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

5 b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the $V_{\text{H}3}$ germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other $V_{\text{H}3}$ germline family members, and a CDR1 that is structurally similar to CDR1s from other $V_{\text{H}3}$ germline family members, and wherein the heavy chain variable region has a mutation at
10 a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue;

 c) has a light chain variable region comprising an amino acid sequence selected from a member of the $V_{\lambda 1}$ germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other $V_{\lambda 1}$ germline
15 family members, and a CDR1 that is structurally similar to CDR1s from other $V_{\lambda 1}$ germline family members, and wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

 In a preferred embodiment, the isolated human antibody, or antigen binding
20 portion thereof, has a mutation in the heavy chain CDR3. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR3. In another embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof,
25 has a mutation in the light chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR1. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR1.

 In another aspect, the invention provides recombinant expression vectors
30 carrying the antibody-encoding nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are

methods of making the antibodies of the invention by culturing the host cells of the invention.

In another aspect, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one
5 additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12.

In another aspect, the invention provides a pharmaceutical composition comprising the antibody or an antigen binding portion thereof, of the invention and a
10 pharmaceutically acceptable carrier.

In another aspect, the invention provides a composition comprising the antibody or an antigen binding portion thereof, and an additional agent, for example, a therapeutic agent.

In another aspect, the invention provides a method for inhibiting human IL-12
15 activity comprising contacting human IL-12 with the antibody of the invention, *e.g.*, J695, such that human IL-12 activity is inhibited.

In another aspect, the invention provides a method for inhibiting human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising administering to the human subject the antibody of the
20 invention, *e.g.*, J695, such that human IL-12 activity in the human subject is inhibited. The disorder can be, for example, Crohn's disease, multiple sclerosis or rheumatoid arthritis.

In another aspect, the invention features a method for improving the activity of an antibody, or an antigen binding portion thereof, to attain a predetermined target
25 activity, comprising:

- a) providing a parent antibody a antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.
- 30 c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;

- 16 -

d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determine if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;

5 e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.

f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.

g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determine if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

25 j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H33B, H52B and L31A are mutated to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

In another aspect, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

5 In one embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity is not further improved by mutagenesis in said phage-display system;

10 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby
15 create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation
20 position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding
25 portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. In another preferred embodiment, the hypermutation positions are selected
30 from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32,

L53 and L93. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

In a more preferred embodiment, the contact positions are selected from the group
5 consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof ;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation
10 position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expressing
15 said panel in an appropriate expression system;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
 - e) evaluating the panel of mutated antibodies, or antigen-binding portions
20 thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained in the antibody;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding
25 portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-
30 crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce

- 20 -

an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, *i.e.* recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, *i.e.* recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, *i.e.* recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment of the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

- 21 -

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
10 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective
15 mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

20 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

25 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred

- 22 -

embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

- 23 -

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained;

until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred

- 24 -

embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer
5 preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof;
10 that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position contact or hypermutation
15 position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- 20 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
25 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.
- f) repeating steps a) through e) for at least one other preferred selective
30 mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained other characteristic, to form combination antibodies, or antigen-binding portions thereof; and

5 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

10 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other
20 characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective
25 mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and

- 26 -

L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues. 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody
5 with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
 - b) selecting an amino acid residue within a complementarity determining region
10 (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
 - c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions
15 thereof;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
 - e) evaluating the panel of mutated antibodies, or antigen-binding portions
20 thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of
25 non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the
30 activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;

- 27 -

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof,
10 thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

15 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent
20 antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

25 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,
30 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in
10 at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,
25 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

30 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

- 29 -

e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

5 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid
10 residues, relative to the parent antibody or antigen-binding portion thereof;
until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

25 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof,
30 thereby identifying an activity enhancing amino acid residue;

- 30 -

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is
5 neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity
10 and not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity and the retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-
15 binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment the invention provides a method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,
25 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

30 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

- 31 -

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other characteristic or property until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding
5 portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;
b) selecting an amino acid residue within a complementarity determining region
10 (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions
15 thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof,
20 relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32,
25 L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity but not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof with at least one retained property or
30 characteristic; and

- 32 -

h) evaluating the activity and the retention of at least one property of characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other characteristics, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity and retained other characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- 33 -

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;
- d) evaluating the activity and retention of at least one other characteristic or property of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
- e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b nor other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other characteristic or property, to form combination antibodies, or antigen-binding portions thereof; and
- g) evaluating the activity and retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- 34 -

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce
5 an antibody with a close to germline immunoglobulin sequence

Brief Description of the Drawings

Figures 1A-1B show the heavy chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12 compared to germline
10 sequences Cos-3/JH3 and Dpl18 Lv1042. Kabat numbering is used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 1C-1D show the light chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12. Kabat numbering is
15 used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 2A-2E show the CDR positions in the heavy chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid
20 substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

Figures 2F-2H show the CDR positions in the light chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid
25 substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

Figure 3 demonstrates the *in vivo* efficacy of the human anti-IL-12 antibody J695, on plasma neopterin levels in cynomolgus monkeys.

Figure 4 shows a graph of mean arthritic score versus days after immunization of mice with collagen, demonstrating that treatment with C17.15 significantly decreases
30 arthritis-related symptoms as compared to treatment with rat IgG.

Detailed Description of the Invention

In order that the present invention may be more readily understood, certain terms are first defined.

The term "activity enhancing amino acid residue" includes an amino acid residue
5 which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a contact, hypermutation or preferred selective mutagenesis position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs. An activity enhancing amino acid residue include, an amino acid residue that improves the
10 binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody, for example, the human IL-12 antibody which inhibits human IL-12.

The term "antibody" includes an immunoglobulin molecule comprised of four
15 polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL)
20 and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to
25 carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or "antibody portion") includes fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hIL-12). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments
30 encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a

- 36 -

disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

- 5 Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv): see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed
- 10 within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the
- 15 same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or non-covalent association of the
- 20 antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')₂
- 25 fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein. Preferred antigen binding portions
- 30 are complete domains or pairs of complete domains.

The term "backmutation" refers to a process in which some or all of the somatically mutated amino acids of a human antibody are replaced with the corresponding germline residues from a homologous germline antibody sequence. The heavy and light chain sequences of the human antibody of the invention are aligned separately with the germline sequences in the VBASE database to identify the sequences with the highest homology. Differences in the human antibody of the invention are returned to the germline sequence by mutating defined nucleotide positions encoding such different amino acid. The role of each amino acid thus identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody; as an example, activity enhancing amino acids identified by the selective mutagenesis approach will not be subject to backmutation. To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. Backmutation may occur at any stage of antibody optimization; preferably, backmutation occurs directly before or after the selective mutagenesis approach. More preferably, backmutation occurs directly before the selective mutagenesis approach.

The phrase "human interleukin 12" (abbreviated herein as hIL-12, or IL-12), as used herein, includes a human cytokine that is secreted primarily by macrophages and dendritic cells. The term includes a heterodimeric protein comprising a 35 kD subunit (p35) and a 40 kD subunit (p40) which are both linked together with a disulfide bridge. The heterodimeric protein is referred to as a "p70 subunit". The structure of human IL-12 is described further in, for example, Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237. The term human IL-12 is intended to include recombinant human IL-12 (rh IL-12), which can be prepared by standard recombinant expression methods.

The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-391 and , Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The Kabat numbering is used herein to indicate the positions of amino acid modifications made in antibodies of the invention. For example, the Y61 anti-IL-12 antibody can be mutated from serine (S) to glutamic acid (E) at position 31 of the heavy chain CDR1 (H31S → E), or glycine (G) can be mutated to tyrosine (Y) at position 94 of the light chain CDR3 (L94G → Y).

The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat *et al.* (See Kabat, *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. The mutations preferably are introduced using the "selective mutagenesis approach" described herein. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. The human antibody can have up to twenty positions replaced with amino acid residues which are not part of the human germline immunoglobulin sequence. In other embodiments, up to ten, up to five, up to three or up to two positions are replaced. In a preferred embodiment, these replacements are within

- 39 -

the CDR regions as described in detail below. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

5 The phrase "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II, below), antibodies isolated from a recombinant, combinatorial human antibody library (described further in Section III, below), antibodies isolated
10 from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor, L.D., *et al.* (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline
15 immunoglobulin sequences (See Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid
20 sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis approach or backmutation or both.

25 An "isolated antibody" includes an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hIL-12 is substantially free of antibodies that specifically bind antigens other than hIL-12). An isolated antibody that specifically binds hIL-12 may bind IL-12 molecules from other species (discussed in further detail below). Moreover,
30 an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody" (or an "antibody that neutralized hIL-12 activity") includes an antibody whose binding to hIL-12 results in inhibition of the biological activity of hIL-12. This inhibition of the biological activity of hIL-12 can be assessed by measuring one or more indicators of hIL-12 biological activity, such as inhibition of human phytohemagglutinin blast proliferation in a phytohemagglutinin blast proliferation assay (PHA), or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3-Interferon-gamma Induction Assay). These indicators of hIL-12 biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see Example 3).

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-hIL-12 antibody that binds to an IL-12 antigen and/or the neutralizing potency of an antibody, for example, an anti-hIL-12 antibody whose binding to hIL-12 inhibits the biological activity of hIL-12, e.g. inhibition of PHA blast proliferation or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3).

The phrase "surface plasmon resonance" includes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 5 and Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term " K_{off} ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term " K_d ", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

The phrase "nucleic acid molecule" includes DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The phrase "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind hIL-12 including "isolated antibodies"), includes a nucleic acid molecule in which the

nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hIL-12, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a
5 VH region of an anti-IL-12 antibody contains no other sequences encoding other VH regions that bind antigens other than IL-12. The phrase "isolated nucleic acid molecule" is also intended to include sequences encoding bivalent, bispecific antibodies, such as diabodies in which VH and VL regions contain no other sequences other than the sequences of the diabody.

10 The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of
15 autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes
20 to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However,
25 the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The phrase "recombinant host cell" (or simply "host cell") includes a cell into which a recombinant expression vector has been introduced. It should be understood
30 that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not,

- 42 -

in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "modifying", as used herein, is intended to refer to changing one or more amino acids in the antibodies or antigen-binding portions thereof. The change can be produced by adding, substituting or deleting an amino acid at one or more positions. 5 The change can be produced using known techniques, such as PCR mutagenesis.

The phrase "contact position" includes an amino acid position of in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the light chain variable region of an antibody which is occupied by an amino acid that contacts antigen in one of the 10 twenty-six known antibody-antigen structures. If a CDR amino acid in any of the 26 known solved structures of antibody-antigen complexes contacts the antigen, then that amino acid can be considered to occupy a contact position. Contact positions have a higher probability of being occupied by an amino acid which contact antigen than non-contact positions. Preferably a contact position is a CDR position which contains an 15 amino acid that contacts antigen in greater than 3 of the 26 structures (>11.5 %). Most preferably a contact position is a CDR position which contains an amino acid that contacts antigen in greater than 8 of the 25 structures (>32%).

The term "hypermutation position" includes an amino acid residue that occupies position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the 20 light chain variable region of an antibody that is considered to have a high frequency or probability for somatic hypermutation during *in vivo* affinity maturation of the antibody. "High frequency or probability for somatic hypermutation" includes frequencies or probabilities of a 5 to about 40% chance that the residue will undergo somatic hypermutation during *in vivo* affinity maturation of the antibody. It should be 25 understood that all ranges within this stated range are also intended to be part of this invention, e.g., 5 to about 30%, e.g., 5 to about 15%, e.g., 15 to about 30%.

The term "preferred selective mutagenesis position" includes an amino acid residue that occupies a position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the light chain variable region which can be considered to be both a 30 contact and a hypermutation position.

The phrase "selective mutagenesis approach" includes a method of improving the activity of an antibody by selecting and individually mutating CDR amino acids at at least one preferred selective mutagenesis position, hypermutation, and/or contact position. A "selectively mutated" human antibody is an antibody which contains a mutation at a position selected using a selective mutagenesis approach. In another embodiment, the selective mutagenesis approach is intended to provide a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region (hereinafter H1, H2, and H3, respectively), or the CDR1, CDR2 or CDR3 of the light chain variable region (hereinafter referred to as L1, L2, and L3, respectively) of an antibody. Amino acid residues may be selected from preferred selective mutagenesis positions, contact positions, or hypermutation positions. Individual amino acids are selected based on their position in the light or heavy chain variable region. It should be understood that a hypermutation position can also be a contact position. In an embodiment, the selective mutagenesis approach is a "targeted approach". The language "targeted approach" is intended to include a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the CDR1, CDR2 or CDR3 of the light chain variable region of an antibody in a targeted manner, *e.g.*, a "Group-wise targeted approach" or "CDR-wise targeted approach". In the "Group-wise targeted approach", individual amino acid residues in particular groups are targeted for selective mutations including groups I (including L3 and H3), II (including H2 and L1) and III (including L2 and H1), the groups being listed in order of preference for targeting. In the "CDR-wise targeted approach", individual amino acid residues in particular CDRs are targeted for selective mutations with the order of preference for targeting as follows: H3, L3, H2, L1, H1 and L2. The selected amino acid residue is mutated, *e.g.*, to at least two other amino acid residues, and the effect of the mutation on the activity of the antibody is determined. Activity is measured as a change in the binding specificity/affinity of the antibody, and/or neutralization potency of the antibody. It should be understood that the selective mutagenesis approach can be used for the optimization of any antibody derived from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells. Preferably, the selective mutagenesis approach is used on antibodies which can not be optimized further using

phage display technology. It should be understood that antibodies from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells can be subject to backmutation prior to or after the selective mutagenesis approach.

5 The term "activity enhancing amino acid residue" includes an amino acid residue which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a preferred selective mutagenesis position, contact position, or a hypermutation position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs.

10 An activity enhancing amino acid residue include, an amino acid residue that improves the binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody, for example, the human IL-12 antibody which inhibits human IL-12.

15 Various aspects of the invention are described in further detail in the following subsections.

I. Human Antibodies that Bind Human IL-12

 This invention provides isolated human antibodies, or antigen-binding portions thereof, that bind to human IL-12. Preferably, the human antibodies of the invention are
20 recombinant, neutralizing human anti-hIL-12 antibodies. Antibodies of the invention that bind to human IL-12 can be selected, for example, by screening one or more human V_L and V_H cDNA libraries with hIL-12, such as by phage display techniques as described in Example 1. Screening of human V_L and V_H cDNA libraries initially identified a series of anti-IL-12 antibodies of which one antibody, referred to herein as
25 "Joe 9" (or "Joe 9 wild type"), was selected for further development. Joe 9 is a relatively low affinity human IL-12 antibody (*e.g.*, a K_{off} of about 0.1 sec^{-1}), yet is useful for specifically binding and detecting hIL-12. The affinity of the Joe 9 antibody was improved by conducting mutagenesis of the heavy and light chain CDRs, producing a panel of light and heavy chain variable regions that were "mixed and matched" and
30 further mutated, leading to numerous additional anti-hIL-12 antibodies with increased affinity for hIL-12 (see Example 1, Table 2 (see Appendix A) and the sequence alignments of Figures 1A-D).

- 45 -

Of these antibodies, the human anti-hIL-12 antibody referred to herein as Y61 demonstrated a significant improvement in binding affinity (*e.g.*, a K_{off} of about 2×10^{-4} sec⁻¹). The Y61 anti-hIL-12 antibody was selected for further affinity maturation by individually mutating specific amino acids residues within the heavy and light chain CDRs . Amino acids residues of Y61 were selected for site-specific mutation (selective mutagenesis approach) based on the amino acid residue occupying a preferred selective mutagenesis position, contact and/or a hypermutation position. A summary of the substitutions at selected positions in the heavy and light chain CDRs is shown in Figures 2A-2H. A preferred recombinant neutralizing antibody of the invention, referred to herein as J695, resulted from a Gly to Tyr substitution at position 50 of the light chain CDR2 of Y61, and a Gly to Tyr substitution at position 94 of the light chain CDR3 of Y61.

Amino acid sequence alignments of the heavy and light chain variable regions of a panel of anti-IL-12 antibodies of the invention, on the lineage from Joe 9 wild type to J695, are shown in Figures 1A-1D. These sequence alignments allowed for the identification of consensus sequences for preferred heavy and light chain variable regions of antibodies of the invention that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1 , on the lineage from Joe 9 to J695. Moreover, the Y61 mutagenesis analysis summarized in Figures 2A-2H allowed for the identification of consensus sequences for heavy and light chain variable regions that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1 that bind hIL-12 on the lineage from Y61 to J695 that encompasses sequences with modifications from Y61 yet that retain good hIL-12 binding characteristics. Preferred CDR, VH and VL sequences of the invention (including consensus sequences) as identified by sequence identifiers in the attached Sequence Listing, are summarized below.

SEQ ID NO :	ANTIBODY CHAIN	REGION	SEQUENCE
1	Consensus Joe 9 to J695	CDR H3	(H/S) -G-S- (H/Y) -D- (N/T/Y)
2	Consensus	CDR L3	Q- (S/T) -Y- (D/E) - (S/R/K) - (S/G/Y) -

- 46 -

	Joe 9 to J695		(L/F/T/S) - (R/S/T/W/H) - (G/P) - (S/T/A/L) - (R/S/M/T/L) - (V/I/T/M/L)
3	Consensus Joe 9 to J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
4	Consensus Joe 9 to J695	CDR L2	(G/Y) - N - (D/S) - (Q/N) - R - P - S
5	Consensus Joe 9 to J695	CDR H1	F-T-F-S- (S/E) - Y-G-M-H
6	Consensus Joe 9 to J695	CDR L1	(S/T) - G - (G/S) - (R/S) - S - N - I - (G/V) - (S/A) - (N/G/Y) - (T/D) - V - (K/H)
7	Consensus Joe 9 to J695	VH	(full VH sequence; see sequence listing)
8	Consensus Joe 9 to J695	VL	(full VL sequence; see sequence listing)
9	Consensus Y61 to J695	CDR H3	H - (G/V/C/H) - (S/T) - (H/T/V/R/I) - (D/S) - (N/K/A/T/S/F/W/H)
10	Consensus Y61 to J695	CDR L3	Q-S-Y- (D/S) - (Xaa) - (G/D/Q/L/F/R/H/N/Y) - T - H - P - A - L - L
11	Consensus Y61 to J695	CDR H2	(F/T/Y) - I - (R/A) - Y - (D/S/E/A) - (G/R) - S - (Xaa) - K - (Y/E) - Y - A - D - S - V - K - G
12	Consensus Y61 to J695	CDR L2	(G/Y/S/T/N/Q) - N - D - Q - R - P - S
13	Consensus Y61 to J695	CDR H1	F-T-F- (Xaa) - (Xaa) - (Y/H) - (G/M/A/N/S) - M - H
14	Consensus Y61 to J695	CDR L1	S-G-G-R-S-N-I-G- (S/C/R/N/D/T) - (N/M/I) - (T/Y/D/H/K/P) - V - K
15	Consensus Y61 to J695	VH	(full VH sequence; see sequence listing)
16	Consensus Y61 to J695	VL	(full VL sequence; see sequence listing)
17	Y61	CDR H3	H-G-S-H-D-N
18	Y61	CDR L3	Q-S-Y-D-R-G-T-H-P-A-L-L
19	Y61	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
20	Y61	CDR L2	G-N-D-Q-R-P-S
21	Y61	CDR H1	F-T-F-S-S-Y-G-M-H
22	Y61	CDR L1	S-G-G-R-S-N-I-G-S-N-T-V-K

- 47 -

23	Y61	VH	(full VH sequence; see sequence listing)
24	Y61	VL	(full VL sequence; see sequence listing)
25	J695	CDR H3	H-G-S-H-D-N
26	J695	CDR L3	Q-S-Y-D-R-Y-T-H-P-A-L-L
27	J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
28	J695	CDR L2	Y-N-D-Q-R-P-S
29	J695	CDR H1	F-T-F-S-S-Y-G-M-H
30	J695	CDR L1	S-G-S-R-S-N-I-G-S-N-T-V-K
31	J695	VH	(full VH sequence; see sequence listing)
32	J695	VL	(full VL sequence; see sequence listing)

Antibodies produced from affinity maturation of Joe 9 wild type were functionally characterized by surface plasmon resonance analysis to determine the K_d and K_{off} rate. A series of antibodies were produced having a K_{off} rate within the range of about 0.1 s^{-1} to about $1 \times 10^{-5} \text{ s}^{-1}$, and more preferably a K_{off} of about $1 \times 10^{-4} \text{ s}^{-1}$ to $1 \times 10^{-5} \text{ s}^{-1}$ or less. Antibodies were also characterized *in vitro* for their ability to inhibit phytohemagglutinin (PHA) blast proliferation, as described in Example 3. A series of antibodies were produced having an IC_{50} value in the range of about $1 \times 10^{-6} \text{ M}$ to about $1 \times 10^{-11} \text{ M}$, more preferably about $1 \times 10^{-10} \text{ M}$ to $1 \times 10^{-11} \text{ M}$ or less.

Accordingly, in one aspect, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a K_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less. In preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-2} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-7} \text{ M}$ or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin

- 48 -

blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-8} M or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-4} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-10} M or less. In even more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} s^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-11} M or less.

The dissociation rate constant (K_{off}) of an IL-12 antibody can be determined by surface plasmon resonance (see Example 5). Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). Surface plasmon analysis can also be performed by immobilizing the analyte (antibodies on a biosensor matrix) and presenting the ligand (recombinant IL-12 in solution). Neutralization activity of IL-12 antibodies, or antigen binding portions thereof, can be assessed using one or more of several suitable *in vitro* assays (see Example 3).

It is well known in the art that antibody heavy and light chain CDRs play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, the invention encompasses human antibodies having light and heavy chain CDRs of Joe 9, as well as other antibodies having CDRs that have been modified to improve the binding specificity/affinity of the antibody. As demonstrated in Example 1, a series of modifications to the light and heavy chain CDRs results in affinity maturation of human anti-hIL-12 antibodies. The heavy and light chain variable region amino acid sequence alignments of a series of human antibodies ranging from Joe 9 wild type to J695 that bind human IL-12 is shown in Figures 1A-1D. Consensus sequence motifs for the CDRs of antibodies can be determined from the sequence alignment (as

summarized in the table above). For example, a consensus motif for the VH CDR3 of the lineage from Joe 9 to J695 comprises the amino acid sequence: (H/S)-G-S-(H/Y)-D-(N/T/Y) (SEQ ID NO: 1), which encompasses amino acids from position 95 to 102 of the consensus HCVR shown in SEQ ID NO: 7. A consensus motif for the VL CDR3
 5 comprises the amino acid sequence: Q-(S/T)-Y-(D/E)-(S/R/K)-(S/G/Y)-(L/F/T/S)-(R/S/T/W/H)-(G/P)-(S/T/A/L)-(R/S/M/T/L-V/I/T/M/L) (SEQ ID NO: 2), which encompasses amino acids from position 89 to 97 of the consensus LCVR shown in SEQ ID NO: 8.

Accordingly, in another aspect, the invention provides an isolated human
 10 antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1×10^{-6} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- 15 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the antibody further comprises a VH CDR2 comprising the amino acid sequence: F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G (SEQ ID NO: 3) (which encompasses amino acids from position 50 to 65 of the consensus
 20 HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR2 comprising the amino acid sequence: (G/Y)-N-(D/S)-(Q/N)-R-P-S (SEQ ID NO: 4) (which encompasses amino acids from position 50 to 56 of the consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

In another preferred embodiment, the antibody further comprises a VH CDR1
 25 comprising the amino acid sequence: F-T-F-S-(S/E)-Y-G-M-H (SEQ ID NO: 5) (which encompasses amino acids from position 27 to 35 of the consensus HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR1 comprising the amino acid sequence: (S/T)-G-(G/S)-(R/S)-S-N-I-(G/V)-(S/A)-(N/G/Y)-(T/D)-V-(K/H) (SEQ ID NO: 6) (which encompasses amino acids from position 24 to 34 of the
 30 consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

- 50 -

In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 7 and a LCVR comprising the amino acid sequence of SEQ ID NO: 8.

Additional consensus motifs can be determined based on the mutational analysis performed on Y61 that led to the J695 antibody (summarized in Figures 2A-2H). As demonstrated by the graphs shown in Figures 2A-2H, certain residues of the heavy and light chain CDRs of Y61 were amenable to substitution without significantly impairing the hIL-12 binding properties of the antibody. For example, individual substitutions at position 30 in CDR H1 with twelve different amino acid residues did not significantly reduce the K_{off} rate of the antibody, indicating that is position is amenable to substitution with a variety of different amino acid residues. Thus, based on the mutational analysis (i.e., positions within Y61 that were amenable to substitution by other amino acid residues) consensus motifs were determined. The consensus motifs for the heavy and light chain CDR3s are shown in SEQ ID NOs: 9 and 10, respectively, consensus motifs for the heavy and light chain CDR2s are shown in SEQ ID NOs: 11 and 12, respectively, and consensus motifs for the heavy and light chain CDR1s are shown in SEQ ID NOs: 13 and 14, respectively. Consensus motifs for the VH and VL regions are shown in SEQ ID NOs: 15 and 16, respectively.

Accordingly, in one aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the antibody further comprises a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and further comprises a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 12.

In another preferred embodiment, the antibody further comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 and further comprises a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

- 51 -

In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 15 and a LCVR comprising the amino acid sequence of SEQ ID NO: 16.

A preferred antibody of the invention, the human anti-hIL-12 antibody Y61, was
5 produced by affinity maturation of Joe 9 wild type by PCR mutagenesis of the CDR3 (as described in Example 1). Y61 had an improved specificity/binding affinity determined by surface plasmon resonance and by *in vitro* neutralization assays. The heavy and light chain CDR3s of Y61 are shown in SEQ ID NOs: 17 and 18, respectively, the heavy and light chain CDR2s of Y61 are shown in SEQ ID NOs: 19 and 20, respectively, and the
10 heavy and light chain CDR1s of Y61 are shown in SEQ ID NOs: 21 and 22, respectively. The VH of Y61 has the amino acid sequence of SEQ ID NO: 23 and the VL of Y61 has the amino acid sequence of SEQ ID NO: 24 (these sequences are also shown in Figures 1A-1D, aligned with Joe9).

Accordingly, in another aspect, the invention features an isolated human
15 antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- 20 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:
25 20.

In another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21 and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

5 In certain embodiments, the full length antibody comprises a heavy chain constant region, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions, and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody
10 heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be an Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment.

Modifications of individual residues of Y61 led to the production of a panel of antibodies shown in Figures 2A-2H. The specificity/binding affinity of each antibody
15 was determined by surface plasmon resonance and/or by *in vitro* neutralization assays.

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- 20 b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In preferred embodiment, the isolated human antibody, or an antigen-binding
25 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533.

In another preferred embodiment, the isolated human antibody, or an antigen-
30 binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light

chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505.

In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

In certain embodiments, the full length antibody comprising a heavy chain constant region such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be a Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment.

A particularly preferred recombinant, neutralizing antibody of the invention, J695, was produced by site-directed mutagenesis of contact and hypermutation amino acids residues of antibody Y61 (see Example 2 and section III below). J695 differs from Y61 by a Gly to Tyr substitution in Y61 at position 50 of the light chain CDR2 and by a Gly to Tyr substitution at position 94 of the light chain CDR3. The heavy and light chain CDR3s of J695 are shown in SEQ ID NOs: 25 and 26, respectively, the heavy and light chain CDR2s of J695 are shown in SEQ ID NOs: 27 and 28, respectively, and the heavy and light chain CDR1s of J695 are shown in SEQ ID NOs: 29 and 30, respectively. The VH of J695 has the amino acid sequence of SEQ ID NO: 31 and the VL of J695 has the amino acid sequence of SEQ ID NO: 32 (these sequences are also shown in Figures 1A-1D, aligned with Joe9).

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

In preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28.

In another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

In certain embodiments, the full length antibody comprises a heavy chain constant region, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be an Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment. -

Additional mutations in the preferred consensus sequences for CDR3, CDR2, and CDR1 of antibodies on the lineage from Joe 9 to J695, or from the lineage Y61 to J695, can be made to provide additional anti-IL-12 antibodies of the invention. Such methods of modification can be performed using standard molecular biology techniques, such as by PCR mutagenesis, targeting individual contact or hypermutation amino acid residues in the light chain and/or heavy chain CDRs-, followed by kinetic and functional analysis of the modified antibodies as described herein (e.g., neutralization assays described in Example 3, and by BIAcore analysis, as described in Example 5).

- 55 -

Accordingly, in another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-6} M or less;
- 5 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no
10 more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of
15 SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no
20 more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

In another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- 25 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:
30 13, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a

- 56 -

heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and

- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

An ordinarily skilled artisan will also appreciate that additional mutations to the CDR regions of an antibody of the invention, for example in Y61 or in J695, can be made to provide additional anti-IL-12 antibodies of the invention. Such methods of modification can be performed using standard molecular biology techniques, as described above. The functional and kinetic analysis of the modified antibodies can be performed as described in Example 3 and Example 5, respectively. Modifications of individual residues of Y61 that led to the identification of J695 are shown in Figures 2A-2H and are described in Example 2.

Accordingly, in another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2

- 57 -

comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and

- 5 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

In another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- 15 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and
- 25 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising
- 30

- 58 -

the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

In yet another embodiment, the invention provides isolated human antibodies, or antigen-binding portions thereof, that neutralize the activity of human IL-12, and at least one additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which do not neutralize the activity of the mouse IL-12.

II Selection of Recombinant Human Antibodies

Recombinant human antibodies of the invention can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*TM phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Kang *et al.* PCT Publication No. WO 92/18619; Winter *et al.* PCT Publication No. WO 92/20791; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

The antibody libraries used in this method are preferably scFv libraries prepared from human VL and VH cDNAs. The scFv antibody libraries are preferably screened using recombinant human IL-12 as the antigen to select human heavy and light chain sequences having a binding activity toward IL-12. To select for antibodies specific for the p35 subunit of IL-12 or the p70 heterodimer, screening assays were performed in the

presence of excess free p40 subunit. Subunit preferences can be determined, for example by, micro-Friguet titration, as described in Example 1.

Once initial human VL and VH segments are selected, "mix and match" experiments, in which different pairs of the selected VL and VH segments are screened for IL-12 binding, are performed to select preferred VL/VH pair combinations (see Example 1). Additionally, to further improve the affinity and/or lower the off rate constant for hIL-12 binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be reselected and rescreened for binding to hIL-12 and sequences that exhibit high affinity and a low off rate for IL-12 binding can be selected. Table 2 (see Appendix A) shows antibodies that displayed altered binding specificity/affinity produced as a result of *in vitro* affinity maturation.

Following selection, isolation and screening of an anti-hIL-12 antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the phage particle(s) (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention (*e.g.*, linked to nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described in further detail in Section IV below.

Methods for selecting human IL-12 binding antibodies by phage display technology, and affinity maturation of selected antibodies by random or site-directed mutagenesis of CDR regions are described in further detail in Example 1.

As described in Example 1, screening of human VL and VH cDNA libraries identified a series of anti-IL-12 antibodies, of which the Joe 9 antibody was selected for further development. A comparison of the heavy chain variable region of Joe 9 with the heavy chain germline sequences selected from the VBASE database, revealed that Joe 9
5 was similar to the COS-3 germline sequence. COS-3 belongs to the V_H3 family of germline sequences.

The V_H3 family is part of the human VH germline repertoire which is grouped into seven families, V_H1 - V_H7 , based on nucleotide sequence homology (Tomlinson *et al.* (1992) *J. Mol. Biol.*, 227, 776-798 and Cook *et al.* (1995) *Immunology Today*, 16,
10 237-242). The V_H3 family contains the highest number of members and makes the largest contribution to the germline repertoire. For any given human V_H3 -germline antibody sequence, the amino acid sequence identity within the entire V_H3 family is high (See *e.g.*, Tomlinson *et al.* (1992) *J. Mol. Biol.*, 227, 776-798 and Cook *et al.* (1995) *Immunology Today*, 16, 237-242). The range of amino acid sequence identity
15 between any two germline VH sequences of the V_H3 family varies from 69-98 residues out of approximately 100 VH residues, (*i.e.*, 69-98% amino acid sequence homology between any two germline VH sequences). For most pairs of germline sequences there is at least 80 or more identical amino acid residues, (*i.e.*, at least 80% amino acid sequence homology). The high degree of amino acid sequence homology between the
20 V_H3 family members results in certain amino acid residues being present at key sites in the CDR and framework regions of the VH chain. These amino acid residues confer structural features upon the CDRs.

Studies of antibody structures have shown that CDR conformations can be grouped into families of canonical CDR structures based on the key amino acid residues
25 that occupy certain positions in the CDR and framework regions. Consequently, there are similar local CDR conformations in different antibodies that have canonical structures with identical key amino acid residues (Chothia *et al.* (1987) *J. Mol. Biol.*, 196, 901-917 and Chothia *et al.* (1989) *Nature*, 342, 877-883). Within the V_H3 family there is a conservation of amino acid residue identity at the key sites for the CDR1 and
30 CDR2 canonical structures (Chothia *et al.* (1992) *J. Mol. Biol.*, 227, 799-817).

- 61 -

The COS-3 germline VH gene, is a member of the V_H3 family and is a variant of the 3-30 (DP-49) germline VH allele. COS-3, differs from Joe9 VH amino acid sequences at only 5 positions. The high degree of amino acid sequence homology between Joe9 VH and COS-3, and between Joe9 VH and the other V_H3 family members also confers a high degree of CDR structural homology (Chothia *et al.* (1992) *J. Mol. Biol.*, 227, 799-817; Chothia *et al.* (1987) *J. Mol. Biol.*, 196, 901-917 and Chothia *et al.* (1989) *Nature*, 342, 877-883).

The skilled artisan will appreciate that based on the high amino acid sequence and canonical structural similarity to Joe 9, other V_H3 family members could also be used to generate antibodies that bind to human IL-12. This can be performed, for example, by selecting an appropriate VL by chain-shuffling techniques (Winter *et al.* (1994) *Annual Rev. Immunol.*, 12, 433-55), or by the grafting of CDRs from a rodent or other human antibody including CDRs from antibodies of this invention onto a V_H3 family framework.

The human V lambda germline repertoire is grouped into 10 families based on nucleotide sequence homology (Williams *et al.* (1996) *J. Mol. Biol.*, 264, 220-232). A comparison of the light chain variable region of Joe 9 with the light chain germline sequences selected from the VBASE database, revealed that Joe 9 was similar to the DPL8 lambda germline. The Joe9 VL differs from DPL8 sequence at only four framework positions, and is highly homologous to the framework sequences of the other V_λ1 family members. Based on the high amino acid sequence homology and canonical structural similarity to Joe 9, other V_λ1 family members may also be used to generate antibodies that bind to human IL-12. This can be performed, for example, by selecting an appropriate VH by chain-shuffling techniques (Winter *et al. Supra*, or by the grafting of CDRs from a rodent or other human antibody including CDRs from antibodies of this invention onto a V_λ1 family framework.

The methods of the invention are intended to include recombinant antibodies that bind to hIL-12, comprising a heavy chain variable region derived from a member of the V_H3 family of germline sequences, and a light chain variable region derived from a member of the V_λ1 family of germline sequences. Moreover, the skilled artisan will appreciate that any member of the V_H3 family heavy chain sequence can be combined with any member of the V_λ1 family light chain sequence.

Those skilled in the art will also appreciate that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the germline may exist within a population (e.g., the human population). Such genetic polymorphism in the germline sequences may exist among individuals within a population due to natural allelic variation. Such natural allelic variations can typically result in 1-5 % variance in the nucleotide sequence of the a gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in germline sequences that are the result of natural allelic variation are intended to be within the scope of the invention.

Accordingly, in one aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the $\text{V}_{\text{H}}3$ germline family, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from a member of the $\text{V}_{\lambda}1$ germline family, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

In a preferred embodiment, the isolated human antibody, or antigen binding has mutation in the heavy chain CDR3.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR3.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the heavy chain CDR2.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR2.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the heavy chain CDR1.

- 63 -

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR1.

An ordinarily skilled artisan will appreciate that based on the high amino acid sequence similarity between members of the V_H3 germline family, or between members of the light chain V_λ1 germline family, that mutations to the germlines sequences can provide additional antibodies that bind to human IL-12. Table 1 (see Appendix A) shows the germline sequences of the V_H3 family members and demonstrates the significant sequence homology within the family members. Also shown in Table 1 are the germline sequences for V_λ1 family members. The heavy and light chain sequences of Joe 9 are provided as a comparison. Mutations to the germline sequences of V_H3 or V_λ1 family members may be made, for example, at the same amino acid positions as those made in the antibodies of the invention (e.g. mutations in Joe 9). The modifications can be performed using standard molecular biology techniques, such as by PCR mutagenesis, targeting individual amino acid residues in the germline sequences, followed by kinetic and functional analysis of the modified antibodies as described herein (e.g., neutralization assays described in Example 3, and by BIAcore analysis, as described in Example 5).

Accordingly, in one aspect, the invention features isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

b) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

An ordinarily skilled artisan will appreciate that based on the high amino acid sequence similarity between Joe 9 and COS-3 heavy chain germline sequence, and between Joe 9 and DPL8 lambda germline sequence, that other mutations to the CDR regions of these germlines sequences can provide additional antibodies that bind to

- 64 -

human IL-12. Such methods of modification can be performed using standard molecular biology techniques as described above.

Accordingly, in one aspect, the invention features isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

5 a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising the COS-3 germline
10 amino acid sequence, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a preferred
15 selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

Due to certain amino acid residues occupying key sites in the CDR and framework regions in the light and heavy chain variable region, structural features are conferred at these regions. In particular, the CDR2 and CDR1 regions are subject to
20 canonical structural classifications. Since there is a high degree of amino acids sequence homology between family members, these canonical features are present between family members. The skilled artisan will appreciate that modifications at the amino acid residues that confer these canonical structures would produce additional antibodies that bind to IL-12. The modifications can be performed using standard molecular biology
25 techniques as described above.

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
30 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

- 65 -

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the V_H3 germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_H3 germline family members, and a CDR1 that is structurally similar to CDR1s from other V_H3 germline family members, and wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue;

c) has a light chain variable region comprising an amino acid sequence selected from a member of the V_L1 germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_L1 germline family members, and a CDR1 that is structurally similar to CDR1s from other V_L1 germline family members, and wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

Recombinant human antibodies of the invention have variable and constant regions which are homologous to human germline immunoglobulin sequences selected from the VBASE database. Mutations to the recombinant human antibodies (*e.g.*, by random mutagenesis or PCR mutagenesis) result in amino acids that are not encoded by human germline immunoglobulin sequences. Also, libraries of recombinant antibodies which were derived from human donors will contain antibody sequences that differ from their corresponding germline sequences due to the normal process of somatic mutation that occurs during B-cell development. It should be noted that if the "germline" sequences obtained by PCR amplification encode amino acid differences in the framework regions from the true germline configuration (*i.e.*, differences in the amplified sequence as compared to the true germline sequence), it may be desirable to change these amino acid differences back to the true germline sequences (*i.e.*, "backmutation" of framework residues to the germline configuration). Thus, the present invention can optionally include a backmutation step. To do this, the amino acid sequences of heavy and light chain encoded by the germline (as found as example in VBASE database) are first compared to the mutated immunoglobulin heavy and light chain framework amino acid sequences to identify amino acid residues in the mutated immunoglobulin framework sequence that differ from the closest germline sequences.

- 66 -

Then, the appropriate nucleotides of the mutated immunoglobulin sequence are mutated back to correspond to the germline sequence, using the genetic code to determine which nucleotide changes should be made. Mutagenesis of the mutated immunoglobulin framework sequence is carried out by standard methods, such as PCR-mediated
5 mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or site-directed mutagenesis. The role of each amino acid identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the
10 final human antibody; as an example, activity enhancing amino acids identified by the selective mutagenesis approach will not be subject to backmutation. Assays to determine the characteristics of the antibody resulting from mutagenesis can include ELISA, competitive ELISA, *in vitro* and *in vivo* neutralization assays and/or (*see e.g.* Example 3) immunohistochemistry with tissue sections from various sources (including human,
15 primate and/or other species).

To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human
20 antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. This would assure that any peptide epitope presented to the immune system by professional antigen presenting cells in a subject treated with the human antibody of the invention would not be foreign but identical to a self-antigen, i.e. the immunoglobulin encoded by that second germline sequence. Backmutation may
25 occur at any stage of antibody optimization; preferably, backmutation occurs directly before or after the selective mutagenesis approach. More preferably, backmutation occurs directly before the selective mutagenesis approach.

III. Modifications to Preferred Selective Mutagenesis Positions, Contact and/or 30 Hypermutation Positions

Typically, selection of antibodies with improved affinities can be carried out using phage display methods, as described in section II above. This can be

- 67 -

accomplished by randomly mutating combinations of CDR residues and generating large libraries containing antibodies of different sequences. However, for these selection methods to work, the antibody-antigen reaction must tend to equilibrium to allow, over time, preferential binding of higher affinity antibodies to the antigen. Selection

5 conditions that would allow equilibrium to be established could not be determined (presumably due to additional non-specific interactions between the antigen and phage particle) when phage display methods were used to improve the affinity of selected anti-IL-12 antibodies, upon attaining a certain level of affinity achieved (*i.e.*, that of antibody Y61). Accordingly, antibodies with even higher affinities could not be selected by

10 phage display methods. Thus, for at least certain antibodies or antigens, phage display methods are limiting in their ability to select antibodies with a highly improved binding specificity/affinity. Accordingly, a method termed Selective Mutagenesis Approach which does not require phage display affinity maturation of antibodies, was established to overcome this limitation and is provided by the invention. Although this Selective

15 Mutagenesis Approach was developed to overcome limitations using the phage display system, it should be noted that this method can also be used with the phage display system. Moreover, the selective mutagenesis approach can be used to improve the activity of any antibody.

To improve the activity (e.g., affinity or neutralizing activity) of an antibody,

20 ideally one would like to mutate every CDR position in both the heavy and light chains to every other possible amino acid residue. However, since there are, on average, 70 CDR positions within an antibody, such an approach would be very time consuming and labor intensive. Accordingly, the method of the invention allows one to improve the activity of the antibody by mutating only certain selected residues within the heavy

25 and/or light chain CDRs. Furthermore, the method of the invention allows improvement in activity of the antibody without affecting other desirable properties of the antibody.

Determining which amino acid residues of an antibody variable region are in contact with an antigen cannot be accurately predicted based on primary sequence or their positions within the variable region. Nevertheless, alignments of sequences from

30 antibodies with different specificities conducted by Kabat *et al.* have identified the CDRs as local regions within the variable regions which differ significantly among antibodies (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-393, , Kabat, E.A., *et al.*

(1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Structural studies have shown that the antigen binding surface is formed by amino acid residues present in the CDRs. Other amino acid residues outside the CDR are also known to play structural
5 roles or be directly involved in antigen binding. Therefore, for each antigen-antibody pair, amino acid residues within and outside of the CDRs may be important.

The sequence alignment studies by Tomlison *et al* identified a number of positions in the heavy and light chain CDR1 and CDR2, and in a portion of the kappa chain CDR3 which are frequent sites of somatic mutation. (Tomlison *et al* (1996) *J. Mol. Biol.* 256: 813-817). In particular, positions H31, H31B, H33, H33B, H52B, H56,
10 H58, L30, L31, L31A, L50, L53, L91, L92, L93 and L94 were identified as frequent sites for somatic mutation. However, this analysis excludes the important heavy chain CDR3 regions, and sections of the light chain CDR3 which are known to lie in the center of an antibody binding site, and potentially provide important interactions with an
15 antigen. Furthermore, Tomlison *et al.* propose that somatic diversity alone does not necessarily predict a role of a specific amino acid in antigen binding, and suggest conserved amino acid residues that contact the antigen, and diverse amino acid residues which do not contact the antigen. This conclusion is further supported by mutational studies on the role of somatic mutations to antibody affinity (*Sharon, (1990), PNAS, 87:4814-7*). Nineteen somatic mutations in a high-affinity anti-p-azophenylarsonate
20 (Ars) antibody were simultaneously replaced with their corresponding germline residues, generating a germline version of the anti-Ars antibody which had a two-hundred fold loss in activity. The full affinity of the anti-Ars antibody could be recovered by restoring only three of the nineteen somatic mutations, demonstrating that
25 many somatic mutations may be permitted that do not contribute to antigen binding activity.

The result can be explained in part by the nature of antibody diversity itself. Immature B-cells may produce initially low affinity antibodies that recognize a number of self or non-self antigens. Moreover, antibodies may undergo in the course of affinity
30 maturation sequence variations that may cause self-reactivity. Hypermutation of such low affinity antibodies may serve to abolish self-reactivity ("negative selection") and increase affinity for the foreign antigen. Therefore, the analysis of primary and

structural data of a large number of antibodies does not provide a method of predicting either (1) the role of somatic hyper-mutation sites in the affinity maturation process versus the process of decreasing affinity towards unwanted antigens, or (2) how a given amino acid contributes to the properties of a specific antigen-antibody pair.

5 Other attempts to address the role of specific amino acid residues in antigen recognition were made by analyzing a number of crystal structures of antigen-antibody complexes (MacCallum *et al.* (1996) *J. Mol. Biol.* 262: 732-745). The potential role of positions located within and outside the CDRs was indicated. Positions in CDRs involved in antigen binding in more than 10 of 26 analyzed structures included H31,
10 H33, H50, H52, H53, H54, H56, H58, H95, H96, H97, H98 and H100 in the heavy chain and L30A, L32, L91, L92, L93, L94, L96 in the light chain. However, the authors noted that prediction of antigen contacts using these and other structural data may over and under predict contact positions, leading to the speculation that a different strategy may have to be applied to different antigens.

15 Pini *et al.* describe randomizing multiple residues in antibody CDR sequences in a large phage display library to rapidly increase antibody affinity (Pini *et al.* (1998) *J. Biol Chem.* 273: 21769-21776). However, the high affinity antibodies discussed by Pini *et al.* had mutations in a total of eight positions, and a reductionary analysis of which changes are absolutely required to improve affinity of the antibody becomes impractical
20 because of the large number of possible combinations to be tested for the smallest number of amino acids required.

 Furthermore, randomizing multiple residues may not necessarily preserve other desired properties of the antibody. Desirable properties or characteristics of an antibody are art-recognized and include for example, preservation of non-cross reactivity, e.g.,
25 with other proteins or human tissues and preservation of antibody sequences that are close to human germline immunoglobulin sequences improvement of neutralization potency. Other desirable properties or characteristics include ability to preserve species cross reactivity, ability to preserve epitope specificity and ability to preserve high expression levels of protein in mammalian cells. The desirable properties or
30 characteristics can be observed or measured using art-recognized techniques including but not limited to ELISA, competitive ELISA, *in vitro* and *in vivo* neutralization assays (*see e.g.* Example 3), immunohistochemistry with tissue sections from different sources

including human, primate or other sources as the need may be, and studies to expression in mammalian cells using transient expression or stable expression.

In addition, the method of Pini et al may introduce more changes than the minimal number actually required to improve affinity and may lead to the antibodies triggering anti-human-antibody (HAMA) formation in human subjects.

Further, as discussed elsewhere, the phage display as demonstrated here, or other related method including ribosome display may not work appropriately upon reaching certain affinities between antibody and antigen and the conditions required to reach equilibrium may not be established in a reasonable time frame because of additional interactions including interactions with other phage or ribosome components and the antigen.

The ordinarily skilled artisan may glean interesting scientific information on the origin of antibody diversity from the teachings of the references discussed above. The present invention, however, provides a method for increasing antibody affinity of a specific antigen-antibody pair while preserving other relevant features or desirable characteristics of the antibody. This is especially important when considering the desirability of imparting a multitude of different characteristics on a specific antibody including antigen binding.

If the starting antibody has desirable properties or characteristics which need to be retained, a selective mutagenesis approach can be the best strategy for preserving these desirable properties while improving the activity of the antibody. For example, in the mutagenesis of Y61, the aim was to increase affinity for hIL-12, and to improve the neutralization potency of the antibody while preserving desired properties. Desired properties of Y61 included (1) preservation of non-cross reactivity with other proteins or human tissues, (2) preservation of fine epitope specificity, i.e. recognizing a p40 epitope preferably in the context of the p70 (p40/p35) heterodimer, thereby preventing binding interference from free soluble p40; and (3) generation of an antibody with heavy and light chain amino acid sequences that were as close as possible to their respective germline immunoglobulin sequences.

In one embodiment, the method of the invention provides a selective mutagenesis approach as a strategy for preserving the desirable properties or characteristics of the antibody while improving the affinity and/or neutralization potency. The term "selective mutagenesis approach" is as defined above and includes a

method of individually mutating selected amino acid residues. The amino acid residues to be mutated may first be selected from preferred selective mutagenesis positions, then from contact positions, and then from hypermutation positions. The individual selected position can be mutated to at least two other amino acid residue and the effect of the mutation both on the desired properties of the antibody, and improvement in antibody activity is determined.

The Selective Mutagenesis approach comprises the steps of:

selecting candidate positions in the order 1) preferred selective mutagenesis positions; 2) contact positions; 3) hypermutation positions and ranking the positions based on the location of the position within the heavy and light chain variable regions of an antibody (CDR3 preferred over CDR2 preferred over CDR1);

individually mutating candidate preferred selective mutagenesis positions, hypermutation and/or contact positions in the order of ranking, to all possible other amino acid residues and analyzing the effect of the individual mutations on the activity of the antibody in order to determine activity enhancing amino acid residues;

if necessary, making stepwise combinations of the individual activity enhancing amino acid residues and analyzing the effect of the various combinations on the activity of the antibodies; selecting mutant antibodies with activity enhancing amino acid residues and ranking the mutant antibodies based on the location and identity of the amino acid substitutions with regard to their immunogenic potential. Highest ranking is given to mutant antibodies that comprise an amino acid sequence which nearly identical to a variable region sequence that is described in a germline database, or has an amino acid sequence that is comparable to other human antibodies. Lower ranking is given to mutant antibodies containing an amino acid substitution that is rarely encountered in either germline sequences or the sequences of other human antibodies. The lowest ranking is given to mutant antibodies with an amino acid substitution that has not been encountered in a germline sequence or the sequence of another human antibody. As set forth above, mutant antibodies comprising at least one activity enhancing amino acid residue located in CDR3 is preferred over CDR2 which is preferred over CDR1. The CDRs of the heavy chain variable regions are preferred over those of the light chain variable region.

The mutant antibodies can also be studied for improvement in activity, *e.g.* when compared to their corresponding parental antibody. The improvement in activity of the mutant antibody can be determined for example, by neutralization assays, or binding specificity/affinity by surface plasmon resonance analysis (see Example 3). Preferably, 5 the improvement in activity can be at least 2-20 fold higher than the parental antibody. The improvement in activity can be at least " x_1 " to " x_2 " fold higher than the parental antibody wherein " x_1 " and " x_2 " are integers between and including 2 to 20, including ranges within the state range, *e.g.* 2-15, *e.g.* 5-10.

The mutant antibodies with the activity enhancing amino acid residue also can be 10 studied to determine whether at least one other desirable property has been retained after mutation. For example, with anti-hIL-12 antibodies testing for, (1) preservation of non-cross reactivity with other proteins or human tissues, (2) preservation of epitope recognition, *i.e.* recognizing a p40 epitope preferably in the context of the p70 (p40/p35) heterodimer, thereby preventing binding interference from free soluble p40; and (3) 15 generation of antibodies with heavy and light chain amino acid sequences that were as close as possible to their respective germline immunoglobulin sequences, and determining which would be least likely to elicit a human immune response based on the number of differences from the germline sequence. The same observations can be made on an antibody having more than one activity enhancing amino acid residues, *e.g.* at 20 least two or at least three activity enhancing amino acid residues, to determine whether retention of the desirable property or characteristic has occurred.

An example of the use of a "selective mutagenesis approach", in the mutagenesis of Y61 is described below. The individual mutations H31S→E, L50→Y, or L94G→Y each improved neutralization activity of the antibody. However, when combination 25 clones were tested, the activity of the combined clone H31S→E + L50→Y + L94G→Y was no better than L50→Y + L94G→Y (J695). Therefore, changing the germline amino acid residue Ser to Glu at position 31 of CDR1 was unnecessary for the improved activity of J695 over Y61. The selective mutagenesis approach therefore, identified the minimal number of changes that contributed to the final activity, thereby reducing the 30 immunogenic potential of the final antibody and preserving other desired properties of the antibody.

- 73 -

Isolated DNA encoding the VH and VL produced by the selected mutagenesis approach can be converted into full length antibody chain genes, to Fab fragment genes as to a scFV gene, as described in section IV. For expression of VH and VL regions produced by the selected mutagenesis approach, expression vectors encoding the heavy and light chain can be transfected into variety host cells as described in detail in section IV. Preferred host cells include either prokaryotic host cells, for example, *E. coli*, or eukaryotic host cells, for example, yeast cells, e.g., *S. cerevisiae*. Most preferred eukaryotic host cells are mammalian host cells, described in detail in section IV.

The selective mutagenesis approach provides a method of producing antibodies with improved activities without prior affinity maturation of the antibody by other means. The selective mutagenesis approach provides a method of producing antibodies with improved affinities which have been subject to back mutations. The selective mutagenesis approach also provides a method of improving the activity of affinity matured antibodies.

The skilled artisan will recognize that the selective mutagenesis approach can be used in standard antibody manipulation techniques known in the art. Examples include, but are not limited to, CDR grafted antibodies, chimeric antibodies, scFV fragments, Fab fragments of a full length antibodies and human antibodies from other sources, e.g., transgenic mice.

Rapid large scale mutational analysis of antibodies include *in vitro* transcription and translation using ribosome display technology (see e.g., Hanes *et al.*, (1997) *Proc. Natl. Acad. Sci.* 94: 4937-4942; Dall Acqua *et al.*, (1998) *Curr. Opin. Struc. Biol.* 8: 443-450; He *et al.*, (1997) *Nucleic Acid Res.* 25: 5132-5134), and U.S. Patent Nos. 5,643,768 and 5,658,754 issued to Kawasaki. The selective mutagenesis approach also provides a method of producing antibodies with improved activities that can be selected using ribosomal display techniques.

In the methods of the invention, antibodies or antigen binding portions thereof are further modified by altering individual positions in the CDRs of the HCVR and/or LCVR. Although these modifications can be made in phage-displayed antibodies, the method is advantageous in that it can be performed with antibodies that are expressed in other types of host systems, such as bacterial, yeast or mammalian cell expression

systems. The individual positions within the CDRs selected for modification are based on the positions being a contact and/or hypermutation position.

Preferred contact positions and hypermutation positions as defined herein are shown in Table 3 (see Appendix A) and their modification in accordance with the method of the invention is described in detail in Example 2. Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred amino acid residues (referred to as "preferred selective mutagenesis positions") are both contact and hypermutation positions and are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

Preferred activity enhancing amino acid residues replace amino acid residues located at positions selected from the group consisting of of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94, and L96. More preferred activity enhancing amino acid residues replace amino acid residues located at positions H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94. Particularly, preferred activity enhancing amino acid residues replace amino acid residues located at positions selected from the group consisting of L50 and L94.

In general, the method of the invention involves selecting a particular preferred selective mutagenesis position, contact and/or hypermutation position within a CDR of the heavy or light chain of a parent antibody of interest, or antigen binding portion thereof, randomly mutagenizing that individual position (e.g., by genetic means using a mutagenic oligonucleotide to generate a "mini-library" of modified antibodies), or mutating a position to specific desired amino acids, to identify activity enhancing amino acid residues expressing, and purifying the modified antibodies (e.g., in a non-phage display host system), measuring the activity of the modified antibodies for antigen (e.g., by measuring k_{off} rates by BIAcore analysis), repeating these steps for other CDR positions, as necessary, and combining individual mutations shown to have improved

activity and testing whether the combination(s) generate an antibody with even greater activity (e.g., affinity or neutralizing potency) than the parent antibody, or antigen-binding portion thereof.

Accordingly, in one embodiment, the invention provides a method for improving
5 the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting in order a 1) preferred selective mutagenesis position, 2) contact position, or 3) hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis
10 position, contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding
15 portions thereof, relative to the parent antibody or antigen-binding portion thereof;
- e) optionally, repeating steps a) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;
- f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies,
20 or antigen-binding portions thereof; and
- g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained. Preferably, the
25 selected antibody or antibodies have an improved activity without loss or with retention of at least one desirable characteristic or property of the parental antibody as described above. The desirable characteristic or property can be measured or observed by the ordinarily skilled artisan using art-recognized techniques.

Preferred contact positions are selected from the group consisting of H30, H31,
30 H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B,

- 76 -

H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

- 5 In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:
- a) providing a parent antibody or antigen-binding portion thereof;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation;
 - 10 c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof,
 - 15 thereby identifying an activity enhancing amino acid residue;
 - e) optionally, repeating steps a) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;
 - f) combining, in the parent antibody, or antigen-binding portion thereof, two individual activity enhancing amino acid residues shown to have improved activity, to
 - 20 form combination antibodies, or antigen-binding portions thereof; and
 - g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative
- 25 to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B,

30 H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32,

- 77 -

H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- 5 a) providing a parent antibody or antigen-binding portion thereof;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby
 - 10 create a panel of mutated antibodies, or antigen-binding portions thereof;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
 - e) optionally, repeating steps a) through d) for at least one other preferred
 - 15 selective mutagenesis position, contact or hypermutation position;
 - f) combining, in the parent antibody, or antigen-binding portion thereof, three individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and
 - g) evaluating the activity of the combination antibodies, or antigen-binding
 - 20 portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the activity enhancing amino acid residue replaces amino acid

25 residues located at positions selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96.

Following mutagenesis of individual selected positions, mutated clones can be sequenced to identify which amino acid residues have been introduced into the selected

30 position in each clone. A small number of clones (e.g., about 24) can be selected for sequencing, which statistically should yield 10-15 unique antibodies, whereas larger

- 78 -

numbers of clones (e.g., greater than 60) can be sequenced to ensure that antibodies with every possible substitution at the selected position are identified.

In one embodiment, contact and/or hypermutation positions within the CDR3 regions of the heavy and/or light chains are first selected for mutagenesis. However, for
5 antibodies that have already been affinity matured *in vitro* by random mutagenesis of the CDR3 regions via phage display selection, it may be preferably to first select contact and/or hypermutation positions within CDR1 or CDR2 of the heavy and/or light chain.

In a more preferred embodiment, preferred selective mutagenesis positions within the CDR3 regions of the heavy and/or light chains are first selected for
10 mutagenesis. However, for antibodies that have already been affinity matured *in vitro* by random mutagenesis of the CDR3 regions via phage display selection, it may be preferably to first select preferred selective mutagenesis positions within CDR1 or CDR2 of the heavy and/or light chain.

In another preferred embodiment, the optimization of a selected antibody by the
15 selective mutagenesis approach is done sequentially as follows: preferred selective mutagenesis positions selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 are mutated first to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization potency (and possibly
20 also for at least one other retained characteristic or property discussed elsewhere). If a mutation of a single preferred selective mutagenesis position does not increase the affinity or neutralization potency at all or sufficiently and if even the combination of multiple activity enhancing amino acids replacing amino acids in preferred selective mutagenesis positions does not result in an combination antibody which meets the target
25 activity (including affinity and/or neutralization potency), additional amino acid residues will be selected for selective mutagenesis from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization potency (and possibly also for at least one other
30 retained characteristic or property discussed elsewhere).

- 79 -

If a mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 does not increase the activity (including affinity and/or neutralization potency) at all or not sufficiently and if even the combination of multiple activity enhancing amino acids replacing amino acids in those
5 positions does not result in an combination antibody which meets the targeted activity (including affinity and/or target neutralization potency), additional amino acid residues will be selected for selective mutagenesis from the group consisting of H33B, H52B, L31A and are mutated to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization
10 potency (and possibly also for at least one other retained characteristic or property discussed elsewhere).

It should be understood that the sequential selective mutagenesis approach may end at any of the steps outline above as soon as an antibody with the desired activity (including affinity and neutralization potency) has been identified. If mutagenesis of the
15 preselected positions has identified activity enhancing amino acids residues but the combination antibody still do not meet the targets set for activity (including affinity and neutralization potency) and/or if the identified activity enhancing amino acids also affect other desired characteristics and are therefore not acceptable, the remaining CDR residues may be subjected to mutagenesis (see section IV).

20 The method of the invention can be used to improve activity of an antibody, or antigen binding portion thereof, to reach a predetermined target activity (e.g. a predetermined affinity and/or neutralization potency, and/or a desired property or characteristic).

Accordingly, the invention provides a method of improving the activity of an
25 antibody, or antigen-binding portion thereof, to attain a predetermined target activity, comprising:

- a) providing a parent antibody a antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91,
30 L92, L93, L94.

- 80 -

c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;

d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determine if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;

e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.

f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.

g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determine if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

- 81 -

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H33B, H52B and L31A are mutated to at least two other amino acid residues to thereby create a
5 third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial
10 activity;

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-binding
15 portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

A number of mutagenesis methods can be used, including PCR assembly, Kunkel (dut-ung-) and thiophosphate (Amersham Sculptor kit) oligonucleotide-directed
20 mutagenesis.

A wide variety of host expression systems can be used to express the mutated antibodies, including bacterial, yeast, baculoviral and mammalian expression systems (as well as phage display expression systems). An example of a suitable bacterial expression vector is pUC119(Sfi). Other antibody expression systems are known in the
25 art and/or are described below in section IV.

The modified antibodies, or antigen binding portions thereof, produced by the method of the invention can be identified without the reliance on phage display methods for selection. Accordingly, the method of the invention is particularly advantageous for improving the activity of a recombinant parent antibody or antigen-binding portion
30 thereof, that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in the phage-display system.

- 82 -

Accordingly, in another embodiment, the invention provides a method for improving the affinity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be
5 further improved by mutagenesis in said phage-display system;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position,
10 contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

15 e) optionally repeating steps b) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

20 g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly
30 preferred contact positions are selected from the group consisting of L50 and L94.

With available methods it is not possible or it is extremely laborious to derive an antibody with increased binding affinity and neutralization potency while retaining other properties or characteristics of the antibodies as discussed above. The method of this invention, however, can readily identify such antibodies. The antibodies subjected to the
5 method of this invention can come from any source.

Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof ;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation
10 position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby
15 create a panel of mutated antibodies, or antigen-binding portions thereof and expressing said panel in an appropriate expression system;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
 - 20 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained in the antibody;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at
25 least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93,
30 L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35

heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and
5 L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

10 In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing
15 p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1)
20 preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

If therefore, the affinity of an antibody for a specific antigen should be improved,
25 but where the phage display (or related system including ribosome display) method is no longer applicable, and other desirable properties or characteristics should be retained, the method of the invention can be used. Accordingly, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

30 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

- 85 -

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

5 c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding
10 portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs
15 to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) optionally, repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

20 g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity of the combination antibodies, or antigen-binding
25 portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained other property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group
30 consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-

crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

5 In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer
10 preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

 In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94
15 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

20 In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to
25 produce an antibody with a close to germline immunoglobulin sequence.

 In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be
30 further improved by mutagenesis in said phage-display system;

- 87 -

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position,
5 contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof
10 thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved
15 activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93,
20 L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

25 In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer
30 preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with
5 other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the
10 group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

15 In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- 20 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis positions, contact or hypermutation position to at least two other amino acid residues to thereby
25 create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- 30 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs

- 89 -

to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) optionally, repeating steps a) through e) for at least one other preferred
5 selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained other characteristic, to form combination antibodies, or antigen-binding portions thereof; and

10 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

15 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
20 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and
25 L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

30 In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94

- 90 -

and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

IV. Modifications of other CDR residues

Ultimately, all CDR residues in a given antibody-antigen pair identified by any means to be required as activity enhancing amino acid residues and/or required directly or indirectly for binding to the antigen and/or for retaining other desirable properties or characteristics of the antibody. Such CDR residues are referred to as "preferred selective mutagenesis positions". It should be noted that in specific circumstances that preferred selective mutagenesis residues can be identified also by other means including co-crystallization of antibody and antigen and molecular modeling.

If the preferred attempts to identify activity enhancing amino acids focussing on the preferred selective mutagenesis positions, contact or hypermutation positions described above are exhausted, or if additional improvements are required, the remaining CDR residues may be modified as described below. It should be understood that the antibody could already be modified in any one or more contact or hypermutation positions according to the embodiments discussed above but may require further improvements. Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,

- 91 -

H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position e.g., to at least two other amino acid residues to thereby create a mutated antibody or a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the mutated antibody or the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the mutated antibody or the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

- 92 -

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other CDR position which is
5 neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity,
10 to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding
15 portion thereof, is obtained.

If the preferred attempts to identify activity enhancing amino acids focussing on the contact or hypermutation positions described above are exhausted, or if additional improvements are required, and the antibody in question can not further be optimized by mutagenesis and phage display (or related ribosome display) methods the remaining
20 CDR residues may be modified as described below. It should be understood that the antibody could already be modified in any one or more preferred selective mutagenesis position, contact or hypermutation positions according to the embodiments discussed above but may require further improvements.

Therefore, in another embodiment, the invention provides a method for
25 improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting a selecting an amino acid residue within a complementarity
30 determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in
10 at least one other property or characteristic, until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

If a single mutagenesis is not sufficient to increase the affinity of the antibody other residues may be included in the mutagenesis. Therefore, in another embodiment,
20 the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

25 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid
30 residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

- 94 -

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position which is neither the
5 position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity,
10 to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity and other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;
until an antibody, or antigen-binding portion thereof, with an improved activity, relative
15 to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce
20 an antibody with a close to germline immunoglobulin sequence

The preferred attempts to identify activity enhancing amino acids focussing on the preferred selective mutagenesis positions, contact or hypermutation positions described may be exhausted, or additional improvements may be required, and it is important to retain other properties or characteristics of the antibody.

25 Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other characteristics, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region
30 (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

- 95 -

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof
5 thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion
10 thereof, with an improved activity and retained other property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35
15 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

25 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof,
30 thereby identifying an activity enhancing amino acid residue;

- 96 -

e.) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other characteristic or property;

e) repeating steps b) through e) for at least one other CDR position which is
5 neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity
10 and not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and the retention of at least one other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-
15 binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained other property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Mutagenesis of the preferred selective mutagenesis position, contact and hypermutation residues may not have increased the affinity of the antibody sufficiently,
20 and mutagenesis and the phage display method (or related ribosome display method) may no longer be useful and at least one other characteristic or property of the antibody should be retained.

Therefore, in another embodiment the invention provides a method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:

25 a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,
30 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

- 97 -

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

- 98 -

d) evaluating the activity and retention of at least one other property or characteristic of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

5 e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least
10 two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and retention of at least one property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity
15 enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

20 V. Expression of Antibodies

An antibody, or antibody portion, of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin
25 light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors
30 into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y.,

(1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

To obtain a DNA fragment encoding the heavy chain variable region of Joe 9 wt or a Joe 9 wt-related antibody, antibodies specific for human IL-12 were screened from human libraries and mutated, as described in section II. Once DNA fragments encoding Joe 9 wt or Joe 9 wt-related VH and VL segments are obtained, mutagenesis of these sequences is carried out by standard methods, such as PCR site directed mutagenesis (PCR-mediated mutagenesis in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or other site-directed mutagenesis methods. Human IL-12 antibodies that displayed a level of activity and binding specificity/affinity that was desirable, for example J695, were further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region and any allotypic variant therein as described in Kabat (*et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a lambda constant region.

10 To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly₄-Ser)₃, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended
20 to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be
25 inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the J695 or J695-related light or heavy chain sequences, the expression
30 vector may already carry antibody constant region sequences. For example, one approach to converting the J695 or J695-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain

- 101 -

constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*, U.S. Patent No. 5,464,758 by Bujard *et al.* and U.S. Patent No. 5,654,168 by Bujard *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216,

4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr⁻ host cells with
5 methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or
10 eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more
15 likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr⁻ CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J.
20 Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the
25 host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be
30 desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light

and heavy chains that is not necessary for binding to hIL-12. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hIL-12 by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are culture to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Antibodies or antigen-binding portions thereof of the invention can be expressed in an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D. *et al.* (1992) Nucl. Acids Res. 20: 6287-6295). Plant cells can also be modified to create transgenic plants that express the antibody or antigen binding portion thereof, of the invention.

In view of the foregoing, another aspect of the invention pertains to nucleic acid, vector and host cell compositions that can be used for recombinant expression of the antibodies and antibody portions of the invention. Preferably, the invention features isolated nucleic acids that encode CDRs of J695, or the full heavy and/or light chain variable region of J695. Accordingly, in one embodiment, the invention features an isolated nucleic acid encoding an antibody heavy chain variable region that encodes the J695 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25.

Preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a J695 heavy chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 27. More preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a J695 heavy chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 29. Even more preferably, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 (the full VH region of J695).

In other embodiments, the invention features an isolated nucleic acid encoding an antibody light chain variable region that encodes the J695 light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. Preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695 light chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 28. More preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695 light chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 30. Even more preferably, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32 (the full VL region of J695).

The invention also provides recombinant expression vectors encoding both an antibody heavy chain and an antibody light chain. For example, in one embodiment, the invention provides a recombinant expression vector encoding:

- a) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 31; and
- b) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 32.

The invention also provides host cells into which one or more of the recombinant expression vectors of the invention have been introduced. Preferably, the host cell is a mammalian host cell, more preferably the host cell is a CHO cell, an NS0 cell or a COS cell. Still further the invention provides a method of synthesizing a recombinant human antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant human antibody of the invention is synthesized. The method can further comprise isolating the recombinant human antibody from the culture medium.

VI. Pharmaceutical Compositions and Pharmaceutical Administration

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05%

- 106 -

polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

In a preferred embodiment, the pharmaceutical composition includes the antibody at a dosage of about 0.01 mg/kg - 10 mg/kg. More preferred dosages of the antibody include 1 mg/kg administered every other week, or 0.3 mg/kg administered weekly.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought

about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which IL-12 activity is detrimental. For example, an anti-hIL-12 antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface

molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. It will be appreciated by the skilled practitioner that when the antibodies of the invention are used as part of a combination therapy, a lower dosage of antibody may be desirable than when the antibody alone is administered to a subject (e.g., a synergistic therapeutic effect may be achieved through the use of combination therapy which, in turn, permits use of a lower dose of the antibody to achieve the desired therapeutic effect).

Interleukin 12 plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements. These diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, atopic dermatitis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Hensch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune

haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired

5 Immunodeficiency Related Diseases, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue

10 disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced

15 interstitial lung disease, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycemia, type B insulin resistance with

20 acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, idiopathic leucopenia, autoimmune neutropenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS,

25 sperm autoimmunity, multiple sclerosis (all subtypes), insulin-dependent diabetes mellitus, sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Takayasu's disease/arteritis, autoimmune thrombocytopenia, idiopathic

30 thrombocytopenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis and vitiligo. The human antibodies,

- 110 -

and antibody portions of the invention can be used to treat autoimmune diseases, in particular those associated with inflammation, including, rheumatoid spondylitis, allergy, autoimmune diabetes, autoimmune uveitis.

Preferably, the antibodies of the invention or antigen-binding portions thereof,
5 are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes mellitus and psoriasis, as described in more detail in section VII.

A human antibody, or antibody portion, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune and inflammatory diseases.

10 Antibodies of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the antibodies of the invention or antigen binding portion thereof can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the
15 additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent which imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included
20 within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations which are part of this invention can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three
25 additional agents if the combination is such that the formed composition can perform its intended function.

Preferred combinations are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other preferred combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be
30 reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the anti-IL-12 antibodies of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody

- 111 -

portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or
5 antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include TNF
10 antagonists like chimeric, humanized or human TNF antibodies, D2E7, (U.S. application serial number 08/599,226 filed February 9, 1996), cA2 (RemicadeTM), CDP 571, anti-TNF antibody fragments (*e.g.*, CDP870), and soluble p55 or p75 TNF receptors, derivatives thereof, (p75TNFR1gG (EnbrelTM) or p55TNFR1gG (Lenercept), soluble IL-13 receptor (sIL-13), and also TNF α converting enzyme (TACE) inhibitors; similarly
15 IL-1 inhibitors (*e.g.*, Interleukin-1-converting enzyme inhibitors, such as Vx740, or IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11, anti-P7s and p-selectin glycoprotein ligand (PSGL). Yet another preferred combination are other key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially preferred are IL-
20 18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another preferred combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2)
25 including antibodies, soluble receptors or antagonistic ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, corticosteroids (oral, inhaled and local
30 injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide,

NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as $\text{TNF}\alpha$ or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors),

5 IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein ligand (PSGL), $\text{TNF}\alpha$ converting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors and the derivatives

10 p75TNFR1gG (EnbrelTM) and p55TNFR1gG (Lenercept), sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and $\text{TGF}\beta$). Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting examples of therapeutic agents for inflammatory bowel disease

15 with which an antibody, or antibody portion, of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipooxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 β monoclonal antibodies; anti-IL-6

20 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4,

25 CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement

30 inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as $\text{TNF}\alpha$ or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein

- 113 -

ligand (PSGL), TNF α converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGF β).

Preferred examples of therapeutic agents for Crohn's disease in which an antibody or an antigen binding portion can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (U.S. application serial number 08/599,226, filed February 9, 1996), cA2 (RemicadeTM), CDP 571, anti-TNF antibody fragments (e.g., CDP870), TNFR-Ig constructs (p75TNFR IgG (EnbrelTM) and p55TNFR IgG (Lenercept)), anti-P7s, p-selectin glycoprotein ligand (PSGL), soluble IL-13 receptor (sIL-13), and PDE4 inhibitors. Antibodies of the invention or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Antibodies of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1 β converting enzyme inhibitors (e.g., Vx740) and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. Antibodies of the invention or antigen binding portions thereof, can be combined with IL-11.

Non-limiting examples of therapeutic agents for multiple sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon- β 1a (Avonex; Biogen); interferon- β 1b (Betaseron; Chiron/Berlex); Copolymer 1 (Cop-1; Copaxone; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4,

- 114 -

CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be

combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin,

mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids

5 such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic

agents, complement inhibitors, adrenergic agents, agents which interfere with signalling

by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or

MAP kinase inhibitors), IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-

selectin glycoprotein ligand (PSGL), TACE inhibitors, T-cell signalling inhibitors such

10 as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-

mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors

and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-

6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10,

IL-13 and TGF β).

15 Preferred examples of therapeutic agents for multiple sclerosis in which the

antibody or antigen binding portion thereof can be combined to include interferon- β , for

example, IFN β 1a and IFN β 1b; copaxone, corticosteroids, IL-1 inhibitors, TNF

inhibitors, and antibodies to CD40 ligand and CD80.

The pharmaceutical compositions of the invention may include a "therapeutically

20 effective amount" or a "prophylactically effective amount" of an antibody or antibody

portion of the invention. A "therapeutically effective amount" refers to an amount

effective, at dosages and for periods of time necessary, to achieve the desired therapeutic

result. A therapeutically effective amount of the antibody or antibody portion may vary

according to factors such as the disease state, age, sex, and weight of the individual, and

25 the ability of the antibody or antibody portion to elicit a desired response in the

individual. A therapeutically effective amount is also one in which any toxic or

detrimental effects of the antibody or antibody portion are outweighed by the

therapeutically beneficial effects. A "prophylactically effective amount" refers to an

amount effective, at dosages and for periods of time necessary, to achieve the desired

30 prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at

an earlier stage of disease, the prophylactically effective amount will be less than the

therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.01-20 mg/kg, more preferably 1-10 mg/kg, even more preferably 0.3-1 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

VII. Uses of the Antibodies of the Invention

Given their ability to bind to hIL-12, the anti-hIL-12 antibodies, or portions thereof, of the invention can be used to detect hIL-12 (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting hIL-12 in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion)

- 116 -

bound to hIL-12 or unbound antibody (or antibody portion), to thereby detect hIL-12 in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials,

5 luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes
10 luminol; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Alternative to labeling the antibody, hIL-12 can be assayed in biological fluids by a competition immunoassay utilizing rhIL-12 standards labeled with a detectable substance and an unlabeled anti-hIL-12 antibody. In this assay, the biological sample,
15 the labeled rhIL-12 standards and the anti-hIL-12 antibody are combined and the amount of labeled rhIL-12 standard bound to the unlabeled antibody is determined. The amount of hIL-12 in the biological sample is inversely proportional to the amount of labeled rhIL-12 standard bound to the anti-hIL-12 antibody.

The Y61 and J695 antibodies of the invention can also be used to detect IL-12
20 from species other than humans, in particular IL-12 from primates. For example, Y61 can be used to detect IL-12 in the cynomolgus monkey and the rhesus monkey. J695 can be used to detect IL-12 in the cynomolgus monkey, rhesus monkey, and baboon. However, neither antibody cross reacts with mouse or rat IL-12 (see Example 3, subsection F).

25 The antibodies and antibody portions of the invention are capable of neutralizing hIL-12 activity *in vitro* (see Example 3) and *in vivo* (see Example 4). Accordingly, the antibodies and antibody portions of the invention can be used to inhibit IL-12 activity, *e.g.*, in a cell culture containing hIL-12, in human subjects or in other mammalian subjects having IL-12 with which an antibody of the invention cross-reacts (*e.g.*
30 primates such as baboon, cynomolgus and rhesus). In a preferred embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one additional primate IL-12

- 117 -

selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12. Preferably, the IL-12 is human IL-12. For example, in a cell culture containing, or suspected of containing hIL-12, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hIL-12 activity in the culture.

In another embodiment, the invention provides a method for inhibiting IL-12 activity in a subject suffering from a disorder in which IL-12 activity is detrimental. IL-12 has been implicated in the pathophysiology of a wide variety of disorders (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996; Morita *et al.* (1998) *Arthritis and Rheumatism*. 41: 306-314; Bucht *et al.*, (1996) *Clin. Exp. Immunol.* 103: 347-367; Fais *et al.* (1994) *J. Interferon Res.* 14:235-238; Parronchi *et al.*, (1997) *Am. J. Path.* 150:823-832; Monteleone *et al.*, (1997) *Gastroenterology*. 112:1169-1178, and Berrebi *et al.*, (1998) *Am. J. Path.* 152:667-672; Parronchi *et al.* (1997) *Am. J. Path.* 150:823-832). The invention provides methods for inhibiting IL-12 activity in a subject suffering from such a disorder, which method comprises administering to the subject an antibody or antibody portion of the invention such that IL-12 activity in the subject is inhibited. Preferably, the IL-12 is human IL-12 and the subject is a human subject. Alternatively, the subject can be a mammal expressing a IL-12 with which an antibody of the invention cross-reacts. Still further the subject can be a mammal into which has been introduced hIL-12 (*e.g.*, by administration of hIL-12 or by expression of an hIL-12 transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an antibody of the invention can be administered to a non-human mammal expressing a IL-12 with which the antibody cross-reacts for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the phrase "a disorder in which IL-12 activity is detrimental" is intended to include diseases and other disorders in which the presence of IL-12 in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which IL-12 activity is detrimental is a disorder in which inhibition of IL-12 activity is expected to alleviate the

- 118 -

symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of IL-12 in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of IL-12 in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-IL-12 antibody as described above. There are numerous examples of disorders in which IL-12 activity is detrimental. In one embodiment, the antibodies or antigen binding portions thereof, can be used in therapy to treat the diseases or disorders described herein. In another embodiment, the antibodies or antigen binding portions thereof, can be used for the manufacture of a medicine for treating the diseases or disorders described herein. The use of the antibodies and antibody portions of the invention in the treatment of a few non-limiting specific disorders is discussed further below:

A. Rheumatoid Arthritis:

Interleukin-12 has been implicated in playing a role in inflammatory diseases such as rheumatoid arthritis. Inducible IL-12p40 message has been detected in synovia from rheumatoid arthritis patients and IL-12 has been shown to be present in the synovial fluids from patients with rheumatoid arthritis (see *e.g.*, Morita *et al.*, (1998) *Arthritis and Rheumatism* 41: 306-314). IL-12 positive cells have been found to be present in the sublining layer of the rheumatoid arthritis synovium. The human antibodies, and antibody portions of the invention can be used to treat, for example, rheumatoid arthritis, juvenile rheumatoid arthritis, Lyme arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis. Typically, the antibody, or antibody portion, is administered systemically, although for certain disorders, local administration of the antibody or antibody portion may be beneficial. An antibody, or antibody portion, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune diseases.

In the collagen induced arthritis (CIA) murine model for rheumatoid arthritis, treatment of mice with an anti-IL-12 mAb (rat anti-mouse IL-12 monoclonal antibody, C17.15) prior to arthritis profoundly suppressed the onset, and reduced the incidence and severity of disease. Treatment with the anti-IL-12 mAb early after onset of arthritis

reduced severity, but later treatment of the mice with the anti-IL-12 mAb after the onset of disease had minimal effect on disease severity.

B. Crohn's Disease

5 Interleukin-12 also plays a role in the inflammatory bowel disease, Crohn's disease. Increased expression of IFN- γ and IL-12 occurs in the intestinal mucosa of patients with Crohn's disease (see *e.g.*, Fais *et al.*, (1994) *J. Interferon Res.* 14: 235-238; Parronchi *et al.*, (1997) *Amer. J. Pathol.* 150: 823-832; Monteleone *et al.*, (1997) *Gastroenterology* 112: 1169-1178; Berrebi *et al.*, (1998) *Amer. J. Pathol.* 152:
10 667-672). Anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, *e.g.*, TNBS induced colitis IL-2 knockout mice, and recently in IL-10 knock-out mice. Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of inflammatory bowel diseases.

15 C. Multiple Sclerosis

Interleukin-12 has been implicated as a key mediator of multiple sclerosis. Expression of the inducible IL-12 p40 message or IL-12 itself can be demonstrated in lesions of patients with multiple sclerosis (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996, Drulovic *et al.*, (1997) *J. Neurol. Sci.* 147: 145-150).
20 Chronic progressive patients with multiple sclerosis have elevated circulating levels of IL-12. Investigations with T-cells and antigen presenting cells (APCs) from patients with multiple sclerosis revealed a self-perpetuating series of immune interactions as the basis of progressive multiple sclerosis leading to a Th1-type immune response. Increased secretion of IFN- γ from the T cells led to increased IL-12 production by
25 APCs, which perpetuated the cycle leading to a chronic state of a Th1-type immune activation and disease (Balashov *et al.*, (1997) *Proc. Natl. Acad. Sci.* 94: 599-603). The role of IL-12 in multiple sclerosis has been investigated using mouse and rat experimental allergic encephalomyelitis (EAE) models of multiple sclerosis. In a relapsing-remitting EAE model of multiple sclerosis in mice, pretreatment with anti-IL-
30 12 mAb delayed paralysis and reduced clinical scores. Treatment with anti-IL-12 mAb at the peak of paralysis or during the subsequent remission period reduced clinical

- 120 -

scores. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate symptoms associated with multiple sclerosis in humans.

D. *Insulin-Dependent Diabetes Mellitus*

5 Interleukin-12 has been implicated as an important mediator of insulin-dependent diabetes mellitus (IDDM). IDDM was induced in NOD mice by administration of IL-12, and anti-IL-12 antibodies were protective in an adoptive transfer model of IDDM. Early onset IDDM patients often experience a so-called "honeymoon period" during which some residual islet cell function is maintained. These
10 residual islet cells produce insulin and regulate blood glucose levels better than administered insulin. Treatment of these early onset patients with an anti-IL-12 antibody may prevent further destruction of islet cells, thereby maintaining an endogenous source of insulin.

15 E. *Psoriasis*

Interleukin-12 has been implicated as a key mediator in psoriasis. Psoriasis involves acute and chronic skin lesions that are associated with a TH1-type cytokine expression profile. (Hamid et al. (1996) J. Allergy Clin. Immunol. 1:225-231; Turka et al. (1995) Mol. Med. 1:690-699). IL-12 p35 and p40 mRNAs were detected in
20 diseased human skin samples. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate chronic skin disorders such psoriasis.

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references, including literature references, issued patents, and published patent applications, as cited
25 throughout this application are hereby expressly incorporated by reference. It should further be understood that the contents of all the tables attached hereto (see Appendix A) are incorporated by reference.

APPENDIX A

121

Table 1 VH3 Family Germline Amino Acid Sequences Numbering according to Kabat (Joe9 VH included for comparison)

seq id	germline	CDR H1	CDR H2
594	dp-29	-----	-----
595	dp-30	-----	-----
596	HC15-7	-----	-----
597	VHD26	-----	-----
598	dp-31	-----	-----
599	dp-32	-----	-----
600	dp-33	-----	-----
601	dp-35	-----	-----
602	VH3-8	-----	-----
603	Y8C-9	-----	-----
604	dp-38	-----	-----
605	L5G2	-----	-----
606	L5G3	-----	-----
607	L5G4	-----	-----
608	L5G6	-----	-----
609	v3-15	-----	-----
610	dp-39	-----	-----
611	dp-40	-----	-----
612	dp-59	-----	-----
613	v3-16p	-----	-----
614	v3-19p	-----	-----
615	v3-13	-----	-----
616	dp-42	-----	-----
617	dp-44	-----	-----
618	dp-45	-----	-----
619	dp-47	-----	-----
620	f1m	-----	-----
621	p1	-----	-----
622	v3-64	-----	-----
623	vh26	-----	-----
624	B25	-----	-----
625	b32e	-----	-----
626	B37	-----	-----
627	B43	-----	-----
628	B48	-----	-----
629	B52	-----	-----
630	B54	-----	-----
631	co3-8	-----	-----
632	dp-46	-----	-----
633	F2H	-----	-----
634	F3	-----	-----
635	F7	-----	-----
636	hv3005	-----	-----
637	P2	-----	-----
638	dp-48	-----	-----
639	dp-58	-----	-----

APPENDIX A

122

Table 1
VH3 Family Germline Amino Acid Sequences
Numbering according to Kabat
(Joe9 VH included for comparison)

SEQ ID	germline	CDR H1	CDR H2
MO:	VH		
640	B1	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
641	B13	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
642	B18	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
643	B26	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
644	B28E	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
645	B29E	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
646	B29H	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
647	B30	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
648	B32H	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
649	cos-3	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
650	dp-19	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
651	dp-50	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
652	P6	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
653	P9E	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
654	v3-30	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
655	v3-33	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH
656	dp-51	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN
657	dp-77	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN
658	HG4	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN
659	v3-21	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN
660	v3-48	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN
661	dp-52	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYV1H
662	cos-6	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYMNH
663	dp-53	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYMNH
664	dp-54	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYMNS
665	dp-87	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYMNH
666	VH3-11	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYMNS
667	Joe9 VH	QVQLVQSGGGGVVQPGRSLRLSCAASGFTFS	SYGMH
668			
669			
670			
671			
672			
673			
674			
675			
676			
677			
678			
679			
680			
681			
682			
683			
684			
685			
686			
687			
688			
689			
690			
691			
692			
693			
694			
695			
696			
697			
698			
699			
700			
701			
702			
703			
704			
705			
706			
707			
708			
709			
710			
711			
712			
713			
714			
715			
716			
717			
718			
719			
720			
721			
722			
723			
724			
725			
726			
727			
728			
729			
730			
731			
732			
733			
734			
735			
736			
737			
738			
739			
740			
741			
742			
743			
744			
745			
746			
747			
748			
749			
750			
751			
752			
753			
754			
755			
756			
757			
758			
759			
760			
761			
762			
763			
764			
765			
766			
767			
768			
769			
770			
771			
772			
773			
774			
775			
776			
777			
778			
779			
780			
781			
782			
783			
784			
785			
786			
787			
788			
789			
790			
791			
792			
793			
794			
795			
796			
797			
798			
799			
800			
801			
802			
803			
804			
805			
806			
807			
808			
809			
810			
811			
812			
813			
814			
815			
816			
817			
818			
819			
820			
821			
822			
823			
824			
825			
826			
827			
828			
829			
830			
831			
832			
833			
834			
835			
836			
837			
838			
839			
840			
841			
842			
843			
844			
845			
846			
847			
848			
849			
850			
851			
852			
853			
854			
855			
856			
857			
858			
859			
860			
861			
862			
863			
864			
865			
866			
867			
868			
869			
870			
871			
872			
873			
874			
875			
876			
877			
878			
879			
880			
881			
882			
883			
884			
885			
886			
887			
888			
889			
890			
891			
892			
893			
894			
895			
896			
897			
898			
899			
900			
901			
902			
903			
904			
905			
906			
907			
908			
909			
910			
911			
912			
913			
914			
915			
916			
917			
918			
919			
920			
921			
922			
923			
924			
925			
926			
927			
928			
929			
930			
931			
932			
933			
934			
935			
936			
937			
938			
939			
940			
941			
942			
943			
944			
945			
946			
947			
948			
949			
950			
951			
952			
953			
954			
955			
956			
957			
958			
959			
960			
961			
962			
963			
964			
965			
966			
967			
968			
969			
970			
971			
972			
973			
974			
975			
976			
977			
978			
979			
980			
981			
982			
983			
984			
985			
986			
987			
988			
989			
990			
991			
992			
993			
994			
995			
996			
997			
998			
999			
1000			

APPENDIX A

123

Table 1 V λ 1 Family Germline Amino Acid Sequences
Numbering according to Kabat.
(Joe9 VL included for comparison)

SEQ ID	gene*	VL	CDR L1	CDR L2	CDR L3
668	1b	DPL5	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGNNY.VS	WYQLPGTAPKLLIY DNNKRP
669	1d	DPL4	QSVLTQPPSV3AAPGQKVTISC	SGSSDMGNYA.VS	WYQLPGTAPKLLIY ENNKRP
670	1c	DPL2	QSVLTQPPSV3AAPGQKVTISC	SGSSDMGNYA.VS	WYQLPGTAPKLLIY ENNKRP
671	1q	DPL3	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGSNT.VN	WYQLPGTAPKLLIY SNNKRP
672	1a	DPL1	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGSNT.VN	WYQLPGTAPKLLIY SNNKRP
673	1f	DPL9	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGSNT.VN	WYQLPGTAPKLLIY SNNKRP
674	1e	DPL8	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGSNT.VN	WYQLPGTAPKLLIY SNNKRP
675		JOE9 VL	QSVLTQPPSV3AAPGQKVTISC	SGSSNIGSNT.VN	WYQLPGTAPKLLIY SNNKRP

*Williams, JMB, 1996, 264, 220-232

APPENDIX A

124

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Joe9 wt	77	SGSYDY	110	QSYDSSLRGSRV	1.00E-01	1.50E-06	1.00E-06	
Joe9 wt IgG1	77	SGSYDY	110	QSYDSSLRGSRV			5.00E-07	
70-1	78	HGSHDN	110	Joe9 wt	1.34 e-2		2.00E-07	
70-1 IgG1	78	HGSHDN	110	Joe9 wt			2.00E-07	
70-2	79	HGSYDY	110	Joe9 wt	3.30E-02		3-5.0E-7	
70-7	80	RRRSNY	110	Joe9 wt	1.29E-01		3-5.0E-7	
70-13	81	SGSIDY	110	Joe9 wt	7.20E-02		3-5.0E-7	
78-34	77	wt	111	QSYDRGFTGSRV	1.64 e-2	2.00E-07	6.00E-07	
78-25	77	wt	112	QSYDSSLRGSRV	5.00E-02			
78-28	77	wt	112	QSYDSSLRGSRV	4.66E-02			
78-35	77	wt	113	QSYDSSLTGSRV	4.99E-02	4.00E-07		
79-1	77	wt	114	QSYDSSLWGSRV		2.00E-07	6.00E-07	
101-14	79	70-2	111	78-34	7.52E-03			
101-9	79	70-2	113	78-35	8.54E-03			
101-19	81	70-13	111	78-34	4.56E-02			
101-8	81	70-13	111	78-34	1.01E-02			
101-4	81	70-13	113	78-35	9.76E-03			
101-5	81	70-13	113	78-35	4.45E-02			
101-11 (12)	78	70-1	111	78-34	4.5 e-3		3.00E-08	
101-11 IgG1	78	70-1	111	78-34		1.60E-09		
26-1 (2,3)	78	70-1	114	79-1	7.4 e-3		6.00E-08	
136-9	82	HGSHDD	115	QTYDISESGSRV	3.20E-03			
136-10	82	HGSHDD	116	QSYDRGFTGSRV	1.40E-03	2.00E-09		
136-14	83	HGSHDN	117	QTYDRGFTGSRV	1.10E-03	3.00E-10	1.00E-07	
136-15	83	HGSHDN	118	QTYDKGFTGSSV	7.4 e-4	1.00E-10	2.00E-09	
136-15 germline	83	HGSHDN	118	QTYDKGFTGSSV	4.60E-04		6.00E-09	
136-16	83	HGSHDN	119	QSYDRFTGSRV	6.10E-04	3.00E-10	5.00E-09	
136-17	83	HGSHDN	120	QSYDRNFTGSRV	2.90E-05	2.00E-09	7.00E-09	
136-18	83	HGSHDN	121	QSYDRGFTGSRV	1.10E-03	8.00E-10		
136-21	83	HGSHDN	122	QSYDNGFTGSRV	4.20E-04	2.00E-09		
136-24	83	HGSHDN	123	QSYDNAVATASKV	8.90E-04	1.00E-09		

APPENDIX A

125

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
101-11	84	TT HGSHDN HGOG	124	QSYDRGETGSRV	4.5x10 ⁻³	2x10 ⁻⁹	2.00E-08	
136-15M1	85	AK	124	QSYDRGETGSRV		4.00E-10		
149-4	86S..	124	1.37x10 ⁻³	8x10 ⁻¹¹	3.00E-09	
149-5	87T	125	QSYDSSLWTRV	1.02x10 ⁻³	1.2x10 ⁻¹⁰	3.00E-09	
149-6	84	124	2.73x10 ⁻³	6x10 ⁻¹⁰	2.00E-09	
149-7	84	126D.....	1.13x10 ⁻³	9x10 ⁻¹⁰	3.00E-09	
149-8	88	K.			2.33x10 ⁻³	3x10 ⁻⁹		
149-9	89	K.H.	127	...E.....M.	3.54x10 ⁻³	1.8x10 ⁻¹⁰		
149-11	90S..	128	...N....A..	1.43x10 ⁻²	2x10 ⁻¹⁰	4.00E-09	
149-12	84			3.73x10 ⁻³	neutralising		
149-13	84			2.22x10 ⁻³	5x10 ⁻¹⁰		
149-14	91	.. .R..N.				1.5x10 ⁻¹⁰	6.00E-09	
	92	TT HGSHDN	124	QSYDRGETGSRV				
156-1	93T	126D.....	5.00E-03			
156-2	93T	129R.....				
156-3	93T	128	...N....A..	9.00E-03			
156-4	93T	127	...E.....SM.				
156-5	93T	130	.T..K.....S.				
156-6	92	126D.....	3.00E-03			
156-7	92	129R.....				
156-8	92	128	...N....A..				
156-9	92	127	...E.....SM.				
156-10	92	130	.T..K.....S.				
156-11	94	.K	126D.....				
156-12	94	.K	129R.....				
156-13	94	.K	128	...N....A..				
156-14	94	.K	127	...E.....SM.				
156-15	94	.K	130	.T..K.....S.				
156-16	93T	124				
156-17	92	125	...SSLW.T..	6.00E-03			
156-18	93T	125	...SSLW.T..				

APPENDIX A

126

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	92	TT HGSHDN	124	QSYDRGFTGSRV	2.9x10 ⁻³			
103-1	95	.. Q.R...	124				
103-2	96	K. R.R...	130	.T..K....S.	7.3x10 ⁻⁴	7.00E-11	1.00E-09	
103-3	97K	124	2.5x10 ⁻³			
103-6			131D..T..	4.5x10 ⁻⁴			
103-7	98D	131D..T..	3.7x10 ⁻⁴	1.40E-10	1.00E-09	
103-8	99	K.	130	.T..K....S.	3.3x10 ⁻⁴	6.00E-11	1.50E-09	
103-14 & 9	100	KT HGSHDN	132	QSYDRGFTGSMV	6.7 e-4	4.00E-11	1.20E-09	
103-8 & 2	100	KT HGSHDN	133	QTYDKGFTGSSV	5.3 e-4		1.50E-09	
103-4	101	TT HGSHDN	134	QSYDRGFTGARV	1.6 e-4	8.60E-11	9.00E-10	
103-152	101	TT HGSHDN	135	QSYERGFTGARV		8.60E-11		
	102	TT SGSDY	136	QSYDRGFTGSRVF				
170-1	102	137FK..	2.35E-03			
170-2	102	138VSAY..	8.80E-04			
170-3	102	139L.VTK..	1.11E-03			
170-4	102	140Y.A....	8.11E-04			
170-7	102	141K..	5.30E-04			
170-11	102	142L.F....	4.40E-04			
170-13	102	143YK..	1.59E-03			
170-15	102	144L.Y.L..	4.43E-03			
170-19	103	.. H..H.N	145DYK..	1.00E-03			
170-21	104	.. H..Q.N	146P.L..	3.89E-03			
170-22	102	147L.....	5.60E-04			
170-23	103	.. H..H.N	148A..W	1.00E-03	2.00E-10		
170-24	104	.. H..Q.N	149Y...	2.80E-04	5.00E-10		
170-35	105	A. H..Q.N	136	1.00E-05			
170-38			150P....	2.10E-04			
170-39			151M.S....	2.79E-03			
170-36	83	HGSHDN	152	QSYDRDSTGSRVF	4.00E-04	2.00E-10		
170-25	106	HGSQDT	153	QSYDSSLRGSRVF	5.00E-04	5.00E-11		

APPENDIX A

127

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	106	SGSYDY	136	QSYDGFSGSRVE				
73-B1	107	SGSYDY	154	H...SD.....	3.25E-03	>1E-8		
73-B2	107	SGSYDY	155	H.SES.....	2.07E-03			
73-B6	107	SGSYDY	156	H...NR.....	2.51E-03	>1E-8		
73-C1	107	SGSYDY	157	H...SR.....	2.71E-03	>1E-8		
73-C2	107	SGSYDY	158SE.....	3.79E-03			
73-C6	107	SGSYDY	159T.....	3.96E-03			
73-D1	107	SGSYDY	160	H...S.....	3.99E-03			
73-D2	107	SGSYDY	161T.....	3.56E-03			
73-D4	107	SGSYDY	162	H...TK.....	5.36E-03			
73-D5	107	SGSYDY	163	H.S.S.....	3.57E-03			
73-E3	107	SGSYDY	164SD.....	4.98E-03			
73-E6	107	SGSYDY	165	H..ES.....	4.17E-03			
73-F3	107	SGSYDY	166APWS.....	7.08E-03			
73-F5	107	SGSYDY	167	...DSD....K...	3.74E-03			
73-G2	107	SGSYDY	168	HTN.S.....	3.98E-03			
73-G3	107	SGSYDY	169	H...TR.....	3.50E-03			
73-G4	107	SGSYDY	170MR.....	6.58E-03			
73-G5	107	SGSYDY	171	H.S.SDS.....	6.01E-03			
73-G6	107	SGSYDY	172	...NTD.....	6.30E-03			
73-H2	107	SGSYDY	173S.....	5.93E-03			
73-F6	107	SGSYDY	174	H...M.....	5.87E-03			
73-H3	107	SGSYDY	175	H...N.....	6.85E-03			
73-C5	107	SGSYDY	176	H.H..D.....	4.84E-03			
73-B7	108	HGSQDN	177	QSYDSSLRGSRV	2.50E-03	7.00E-09		

APPENDIX A

128

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
M2 A2	83	HGSHDN	136	<u>QSYDRGFTGSRVF</u>				
M2 A4	83	HGSHDN	178IH.....	4.00E-02			
M2 A5	83	HGSHDN	179S..P.....	8.49E-03			
M2 B1	83	HGSHDN	180I..S.....	4.01E-02			
M2 B3	83	HGSHDN	181S..L.....	7.97E-03			
M2 B4	83	HGSHDN	182I..M.....	4.60E-02			
M2 B5	83	HGSHDN	183I..L.....	4.42E-02			
M2 B6	83	HGSHDN	184S..V.....	8.38E-03			
M2 C2	83	HGSHDN	185L..A.....	2.81E-02			
M2 C3	83	HGSHDN	186S..L.....	4.85E-02			
M2 C4	83	HGSHDN	187T..L.....	4.62E-02			
M2 D1	83	HGSHDN	188S..L.....	8.16E-03			
M2 D2	83	HGSHDN	189TAL.....	4.71E-02			
M2 D3	83	HGSHDN	190IR.....	3.71E-02			
M2 D4	83	HGSHDN	191IRS.....	3.85E-02			
M2 D5	83	HGSHDN	192NRL.....	3.33E-02			
M2 D6	83	HGSHDN	193ETS.....	5.81E-02			
M2 E1	83	HGSHDN	194SSS.....	5.18E-02			
M2 E2	83	HGSHDN	195S...A....	5.01E-02			
M2 E6	83	HGSHDN	196T...K....	5.32E-02			
M2 F1	83	HGSHDN	197SDV.....	9.77E-03			
M2 H5	83	HGSHDN	198A.....	6.16E-02			
					9.90E-03			

APPENDIX A

129

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
A5	83	HGSHDN	124	OSYDRGFTGSRV				
A12	83	HGSHDN	199THPSML	1.12E-03			
A4	83	HGSHDN	200TTPRPM	1.43E-03			
A6	83	HGSHDN	201RNPALT	1.47E-03			
A10	83	HGSHDN	202THPWLH	1.87E-03			
A11	83	HGSHDN	203NSPATV	1.87E-03			
C2	83	HGSHDN	204TFPSPQ	2.07E-03			
A8	83	HGSHDN	205LNPSAT	2.23E-03			
B8	83	HGSHDN	206KSNKML	2.37E-03			
C6	83	HGSHDN	207HTAHLV	2.40E-03			
A3	83	HGSHDN	208QTPSIT	2.42E-03			
B11	83	HGSHDN	209YPRNIL	2.51E-03			
B5	83	HGSHDN	210ITPGLA	2.95E-03			
C10	83	HGSHDN	211QPHAVL	3.04E-03			
C4	83	HGSHDN	212NSPIPT	3.10E-03			
C3	83	HGSHDN	213TPNNSF	3.23E-03			
B2	83	HGSHDN	214S.VDPGPY	3.34E-03			
A2	83	HGSHDN	215RPRHAL	3.61E-03			
C5	83	HGSHDN	216PYHPIR	3.80E-03			
A7	83	HGSHDN	217PHTQPT	3.91E-03			
C9	83	HGSHDN	218HNNFSP	3.95E-03			
B3	83	HGSHDN	219PHTLPH	3.97E-03			
C8	83	HGSHDN	220TPSYPT	4.12E-03			
B7	83	HGSHDN	221S.TSNLLP	5.36E-03			
A1	83	HGSHDN	222DSNHDH	5.45E-03			
C7	83	HGSHDN	223LPRLTH	5.66E-03			
C12	83	HGSHDN	224IPTSYL	5.83E-03			
B10	83	HGSHDN	225LRVQAP	5.85E-03			
B6	83	HGSHDN	226LSD3PL	6.04E-03			
A9	83	HGSHDN	227S.SLRRIL	7.58E-03			
B9	83	HGSHDN	228PARTSP	7.98E-03			
			229RAHPQ	8.66E-03			

APPENDIX A

130

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
177-D7	83	HGSHDN	124	QSYDRGTGSRV				
177-G6	83	HGSHDN	230TOPABI	4.07E-04			
177-D9	83	HGSHDN	231THPTMI	5.50E-04			
177-C6	83	HGSHDN	232RIPABT	6.32E-04			
177-H5	83	HGSHDN	233THPVPA	7.94E-04			
177-H9	83	HGSHDN	234SBPIPA	1.32E-03			
177-H10	83	HGSHDN	235THPVPA	1.58E-03			
144-F1	83	HGSHDN	236THPTMY	3.44E-03			
43-E3	83	HGSHDN	237HHYTFE	5.80E-04			
43-E9	83	HGSHDN	238SHPAAE	8.00E-04			
43-G2	83	HGSHDN	239TIPSIE	8.00E-04			
43-G3	83	HGSHDN	240SSPAIM	7.00E-04			
31-A6	83	HGSHDN	241IWPENLN	9.00E-04			
31-B5	83	HGSHDN	242THPNLN	5.00E-04			
			243THPSIS	5.00E-04			
Y17	83	HGSHDN	124	QSYDRGTGSRV				
Y19	83	HGSHDN	244	QSYDRGSAPMIN	8.90E-05	4.50E-10	>1E-8	
Y38	83	HGSHDN	245	QSYDRGHPAMS	2.26E-04	3.00E-11	>1E-8	
Y45	83	HGSHDN	246THPSIT	5.08E-04	5.50E-11	2.60E-09	
Y61	83	HGSHDN	247TDPAlV	6.17E-04	4.00E-11	4.30E-09	
Y61 IgG	83	HGSHDN	248THPALL	2.75 e-4	4E-11	1.40E-10	
Y61 IgG germline	83	HGSHDN	248THPALL	1.50E-04	1.60E-11	1.30E-10	
Y139	83	HGSHDN	248THPALL	1.50E-04	1.60E-11	1.30E-10	1.60E-10
Y139 IgG1	83	HGSHDN	249SHPALT	5.92E-04	3E-11	4.50E-10	
Y174	83	HGSHDN	249SHPALT			1.00E-09	
Y177	83	HGSHDN	250TTPAPE	7.55E-04	6E-11	2.00E-09	
A5	83	HGSHDN	251SHPTLI	6.61E-04	5E-11	1.00E-09	
A12	83	HGSHDN	252THPSML	4.50E-04	6.60E-11		
D9	83	HGSHDN	253TTPRPM	5.57E-04	2.50E-10		
G6	83	HGSHDN	254RLPAQT	8.21E-04	3.5E-09	>>	
G6 IgG1	83	HGSHDN	255THPLTI	5.08E-04	1E-10	1.00E-09	
C6	83	HGSHDN	255THPLTI			1.00E-09	
Y55	83	HGSHDN	256	QSYDRGTGTPSIT	1.07E-03	3.5E-10	1.00E-08	
			257	QSYDRGTGFMQY	1.06E-03	1.40E-10	>1E-8	

APPENDIX A

131

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
A4	83	HGSHDN	258	QSYDRGRNPALT	6.30E-04	2.50E-10		
A03	83	HGSHDN	259	QSYDRGTHPLTM	3.04E-04	3.00E-11	4.00E-10	
A03 IgG1	83	HGSHDN	260	QSYDRGTHPLTM	3.04 e-4	2.90E-11	3.80E-10	
A03 IgG germline	83	HGSHDN	260	QSYDRGTHPLTM	2.50E-04	3.50E-11	1.75E-10	
99-B11	83	HGSHDN	261	QSYDSGYTGSRV	5.40E-03			
99-C11	83	HGSHDN	262	QSYDSGFTGSRV	5.70E-03			
99-H4	83	HGSHDN	263	QSYDSRFTGSRV	4.80E-03			
99-E9	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-H7	83	HGSHDN	264	QSYPDGTPASRV	3.30E-03			
99-H11	83	HGSHDN	265	QSYSTHMPISRV	4.90E-03			
99-F6	83	HGSHDN	266	QSYDSGSTGSRV	4.90E-03			
99-F7	83	HGSHDN	267	QSYNSYPIISRV	4.80E-03			
99-F8	83	HGSHDN	268	QSYIRAPQV	3.70E-03			
99-F11	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-G7	83	HGSHDN	269	QSYLKSRFTGSRV	4.80E-03			
99-G11	83	HGSHDN	270	QSYDSRFTGSRV	4.30E-03			
L3.3R3M-B1	83	HGSHDN	124	QSYDRGFTGSRV				
L3.3R3M-B3	83	HGSHDN	271FTGSMV	5.46E+00			
L3.3R3M-C6	83	HGSHDN	272FTGSMV	5.51E+00			
L3.3R3M-F9	83	HGSHDN	273FTGFDG	6.17E+00			
L3.3R3M-G8	83	HGSHDN	274TAPALS	4.99E+00			
L3.3R3M-G8	83	HGSHDN	275SYPALR	5.55E+00			
L3.3R3M-H6	83	HGSHDN	276NWPNSN	5.69E+00			
L3.3R3M-H10	83	HGSHDN	277TAPSLI	5.35E+00			
L3.3R3M-A3	83	HGSHDN	278FTGSMV	5.37E+00			
L3.3R3M-F8	83	HGSHDN	279TTPRIR	4.99E+00			
L3.3R3M-G1	83	HGSHDN	280FTGSMV	4.21E+00			
L3.3R3M-G7	83	HGSHDN	281FTGSMV	4.24E+00			
L3.3R3M-H11	83	HGSHDN	282MIPALT	3.95E+00			

APPENDIX A

132

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Y61-L94N	109	CKT HGSHDN	283	QSYDRNTHPALL			8.00E-11	
Y61-L94F	109	CKT HGSHDN	284	QSYDRFTHPALL			6.00E-11	
Y61-L94Y	109	CKT HGSHDN	285	QSYDRYTHPALL		2.00E-11	2.00E-11	
Y61-L94Y IgG	109	CKT HGSHDN	285	QSYDRYTHPALL	1.27E-04	6.00E-11	5.00E-11	
Y61-L50Y	109	CKT HGSHDN	286	QSYDRGTHPALL		2.00E-11		4.00E-11
Y61-L50Y* IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	6.98E-05		2.00E-11	2.00E-11
Y61-L50Y-H31E** IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	2.99E-05		6.00E-11	2.00E-11
Y61-L50Y-H31E- L94Y** IgG	109	CKT HGSHDN	287	QSYDRYTHPALL	4.64E-05		1.00E-11	1.00E-11
J695 (Y61-L94Y- L50Y IgG*)	109	CKT HGSHDN	287	QSYDRYTHPALL	5.14E-05	5.00E-11	1.00E-11	5.00E-12

*CDR L2: L50G to Y

**CDR L2: L50G to Y; CDR H1: H31S to E

APPENDIX A

133

Table 3									
Kabat Number	CDR H1				CDR H2				CDR H3
	27	28	29	30	31	32	33	34	35
Y61 VH	F	T	F	S	S	Y	G	M	H
Contact Positions	x	x	x	x	x	x	x	x	x
Hypermutation Positions	x	x	x						
Kabat number	CDR L1				CDR L2				CDR L3
	24	25	26	27	27A	27B	28	29	30
Y61 VL	S	G	G	R	S	N	I	G	S
Contact Positions	x	x	x	x	x	x	x	x	x
Hypermutation Positions	x	x	x						
x contact and/or hypermutation position x contact and/or hypermutation position mutated in Y61									

APPENDIX A

134

Table 4. Neutralization Activity in the Presence of Excess Free IL-12 p40					
SEQ ID NO:	Clone	PHA assay IC50 (M) p70:p40 1:0	PHA assay IC50 (M) p70:p40 1:20	PHA assay IC50 (M) p70:p40 1:50	
VH: 47	136-15	2.00E-09	5.00E-09	4.00E-09	
VL: 48					
VH: 51	149-5	6.50E-09	7.00E-09	4.00E-09	
VL: 52					
VH: 53	149-6	9.00E-10	1.00E-09	1.00E-09	
VL: 54					
VH: 84	149-7	3.50E-09	2.50E-09	4.00E-09	
VL: 126					
VH: 23	Y61 IgG	1.80E-10		1.80E-10	
VL: 24					
VH: 65	A03 IgG1	2.50E-10		2.20E-10	
VL: 66					
VH: 31	J695	1.00E-11		3.50E-11	
VL: 32					

EXAMPLES

EXAMPLE 1: Isolation of Anti-IL-12 Antibodies

5 A. Screening for IL-12 binding antibodies

Antibodies to hIL-12 were isolated by screening three separate scFv phage display libraries prepared using human VL and VH cDNAs from mRNA derived from human tonsils (referred to as scFv 1), tonsil and peripheral blood lymphocytes (PBL) (referred to as scFv 2), and bone marrow-derived lymphocytes (referred to as
10 BMDL). Construction of the library and methods for selection are described in Vaughan et al. (1996) Nature Biotech. 14: 309-314.

The libraries were screened using the antigens, human IL-12 p70 subunit, human IL-12 p40 subunit, chimaeric IL-12 (mouse p40/human p35), mouse IL-12, biotinylated human IL-12 and biotinylated chimaeric IL-12. IL-12 specific antibodies were selected
15 by coating the antigen onto immunotubes using standard procedures (Marks et al., (1991) J. Mol. Biol. 222: 581-597). The scFv library 2 was screened using either IL-12, or biotinylated-IL-12, and generated a significant number of IL-12 specific binders. Five different clonotypes were selected, determined by BstN1 enzymatic digestion patterns, and confirmed by DNA sequencing. The main clonotypes were
20 VHDP58/VLDPL11, VHDP77/VLDPK31, VHDP47/VL and VHDP77/VLDPK31, all of which recognized the p40 subunit of IL-12.

Screening of the BMDL library with IL-12 p70 generated 3 different clonotypes. Two of these were found to be cross-reactive clones. The dominant clone was sequenced and consisted of VHDP35/VLDP. This clone recognizes the p40 subunit of
25 IL-12. Screening of the scFv library 1, using IL-12 p70, did not produce specific IL-12 antibodies.

In order to identify IL-12 antibodies which preferentially bind to the p70 heterodimer or the p35 subunit of IL-12, rather than the p40 subunit, the combined scFv 1 + 2 library, and the BMDL library were used. To select IL-12 antibodies that
30 recognized the p70 heterodimer or p35 subunit, phage libraries were preincubated and selected in the presence of free p40. Sequencing of isolated clones revealed 9 different antibody lineages. Subunit preferences were further analyzed by 'micro-Friguet'

- 136 -

titration. The supernatant containing scFv was titrated on biotin-captured IL-12 in an ELISA and the ED₅₀ determined. The concentration of scFv producing 50% ED was preincubated with increasing concentrations of free p70 or p40 (inhibitors). A decrease in the ELISA signal on biotin-IL-12 coated plates was measured and plotted against the concentration of free p70 or p40. This provided the IC₅₀ for each clone with respect to p70 and p40. If the titrations for both subunits overlaps, then the scFv binds to both p40 and p70. Any variation from this gives the degree of preference of p70 over p40.

B. Affinity Maturation of Antibody Lineage Specific for IL-12 (Joe 9)

The clones were tested for their ability to inhibit IL-12 binding to its receptor in an IL-12 receptor binding assay (referred to as RBA), and for their ability to inhibit IL-12 induced proliferation of PHA stimulated human blast cells (PHA assay), described in Example 3. Clone Joe 9 had the lowest IC₅₀ value in both the RBA and the PHA assay, with an IC₅₀ value of 1×10^{-6} M in both assays. In addition the heavy chain variable region (VH) of Joe 9 had the least number of changes compared to the closest germline sequence COS-3, identified from the VBASE database. Table 1 (see Appendix A) shows the V_H3 family of germline sequences, of which COS-3 is a member, as well as members of V_L1 family of germline sequences. Therefore, Joe 9 was selected for affinity maturation. The amino acids sequences of VH and VL of the Joe9 wild type (Joe9 wt) antibody are shown in Figure 1A-1D.

In order to increase the affinity of Joe 9, various mutations of the complementarity determining region 3 (CDR3) of both the heavy and light chains were made. The CDR3 variants were created by site-directed PCR mutagenesis using degenerate oligonucleotides specific for either the heavy chain CDR3 (referred to as "H3") or the light chain CDR3 (referred to as "L3"), with an average of three base substitutions in each CDR3 (referred to as "spike"). PCR mutagenesis of the heavy chain CDR3 was performed using the degenerate heavy chain oligonucleotide

5'TGTCCCTTGGCCCCA(G)(T)(A)(G)(T)(C)(A)(T)(A)(G)(C)(T)(C)(C)(A)(C)(T)

GGTCGTACAGTAATA 3' (SEQ ID NO: 580), and oligonucleotide pUC Reverse Tag
GAC ACC TCG ATC AGC GGA TAA CAA TTTCAC ACA GG (SEQ ID NO: 581)

- 137 -

to generate a repertoire of heavy chain CDR3 mutants. The parent light chain was amplified using Joe 9 reverse oligonucleotide (5'TGG GGC CAA GGG ACA3' (SEQ ID NO:582) and the fdteteseq 24+21 oligonucleotide (5'-ATT CGT CCT ATA CCG TTC TAC TTT GTC GTC TTT CCA GAC GTT AGT-3' (SEQ ID NO: 583).

5 Complementarity between the two PCR products was used to drive annealing of the two fragments in a PCR assembly reaction and the full length recombined scFv library was amplified with pUC Reverse Tag (SEQ ID NO: 581) and fdTag 5'-ATT CGT CCT ATA CCG TTC-3' (SEQ ID NO: 584). PCR mutagenesis of the light chain was performed using the light chain oligonucleotide containing a mixture of all four

10 nucleotides

5'GGTCCCAGTTCCGAAGACCCTCGAACC(C)(C)(T)(C)(A)(G)(G)(C)(T)
(G)(C)(T)(G)(T)(C)ATATGACTGGCAGTAATAGTCAGC 3' (SEQ ID NO: 585), and Joe 9 reverse oligonucleotide 5'TGG GGC CAA GGG ACA3' (SEQ ID NO: 586)

to produce a repertoire of light chain CDR3 mutants. The parent heavy chain was
15 amplified with pUC Reverse Tag (SEQ ID NO: 581) and HuJH3FOR oligonucleotide 5'TGAAGAGACGGTGACCATTTGTCCC3' (SEQ ID NO: 587). Complementarity between the two PCR products was used to drive annealing of the two fragments in a PCR assembly reaction and the full length recombined scFv library was amplified with Reverse Tag GAC ACC TCG ATC AGC G (SEQ ID NO: 588) and HuJλ 2-3 FOR
20 NOT oligonucleotide 5'GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC 3' (SEQ ID NO: 589).

Heavy chain CDR3 mutants were selected using 1 nM biotinylated IL-12, and washed for 1 h at room temperature in PBS containing free IL-12 or p40 at a concentration of 7 nM. Clones were analyzed by phage ELISA and those that bound to
25 IL-12 were tested in BIAcore kinetic binding studies using a low density IL-12 chip (see procedure for BIAcore analysis in Example 5). Generally, BIAcore analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). The system
30 utilizes the optical properties of SPR to detect alterations in protein concentrations within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and

- 138 -

specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units (RU) and are displayed with respect to time along the y-axis of a sensorgram. To determine the off rate (k_{off}), on rate (k_{on}), association rate (K_a) and dissociation rate (K_d) constants, BIAcore kinetic evaluation software (version 2.1) was used. Clones that demonstrated an improvement in the k_{off} rate were analyzed by neutralization assays which included inhibition by antibody of IL-12 binding to its receptor (RBA assay), inhibition of IL-12-induced proliferation in PHA stimulated human blast cells (PHA assay), and inhibition of IL-12-induced interferon gamma production by human blast cells (IFN gamma assay). A summary of the dissociation rates and/or IC_{50} values from neutralization assays of heavy chain CDR3 spiked clones 70-1 through 70-13 is presented in Table 2 (see Appendix A). Clone 70-1 displayed a k_{off} rate that was better than the parent Joe 9 clone, and had the lowest IC_{50} value of 2.0×10^{-7} M. Therefore clone 70-1 was selected for conversion to complete IgG1.

Light chain CDR3 mutants were selected using 1 nM biotin-IL-12 and washed with PBS containing 7 nM free p40. Clones were screened in phage ELISA and those that bound to IL-12 were tested in BIAcore binding analysis using low density IL-12 chips. Clones that displayed an off rate which was better than the parent Joe 9 clone were tested in neutralization assays which measured either, inhibition of IL-12 receptor binding, or inhibition of PHA blast cell proliferation. A summary of the dissociation rates and/or IC_{50} values from neutralization assays of light chain CDR3 mutant clones, 78-34 through 79-1, is presented in Table 2 (see Appendix A).

Based on the k_{off} rate, clones 78-34 and 78-35 displayed an improved k_{off} rate compared to the parent Joe 9. Both of these clones were selected for combination analysis with heavy chain mutants.

C. Combination Clones

Mutant light and heavy chain clones that exhibited the best binding characteristics were used for combination and assembly of scFvs. Mutant clones with improved potency characteristics were combined by PCR overlap extension and pull-

through of the mutated VH and VL segments as described above. Clones 101-14 through 26-1, shown in Table 2 (see Appendix A), were produced from the combination of heavy chain mutants (70-2, 70-13 and 70-1) with light chain mutants (78-34, 78-35 and 79-1). The k_{off} rates and/or IC_{50} values from neutralization assays for these clones are presented in Table 2.

BIAcore binding analysis identified clone 101-11, produced from the combination of the heavy chain CDR3 mutant clone 70-1 with the light chain CDR3 mutant clone 78-34, as having an off rate of 0.0045 s^{-1} . This k_{off} rate was a significant improvement compared to the k_{off} rates for either the heavy chain CDR3 mutant clone 70-1 (0.0134 s^{-1}), or for the light chain CDR3 mutant clone 78-34 (0.0164 s^{-1}) alone. Furthermore, clone 101-11 showed a significant improvement in neutralization assays. Accordingly, clone 101-11 was selected for affinity maturation as described below.

D. Affinity maturation of clone 101-11

Further affinity maturation of clone 101-11 consisted of repeat cycles of PCR mutagenesis of both the heavy and light chain CDR3s of 101-11 using spiked oligonucleotide primers. The clones were selected with decreasing concentrations of biotinylated IL-12 (bio-IL-12). The binding characteristics of the mutated clones was assessed by BIAcore binding analysis and RBA, PHA neutralization assays. The k_{off} rates and/or IC_{50} values for clones 136-9 through 170-25 are presented in Table 2 (see Appendix A). Clone 103-14 demonstrated an improved IC_{50} value in both the receptor binding assay and the PHA blast assay. Clone 103-14 also demonstrated a low k_{off} rate, and accordingly was selected for further affinity maturation.

E. Generation and Selection of Randomized Libraries of Clone 103-14 Light CDR3

The light chain CDR3 of clone 103-14 (QSYDRGFTGSMV (SEQ ID NO: 590)) was systematically randomized in 3 segments using 3 different libraries as outlined below, where X is encoded by a randomized codon of sequence NNS with N being any nucleotide and S being either deoxycytosine or deoxyguanine.

L3.1=XXXXXXXXFTGSMV (SEQ ID NO: 591)

L3.2= QSYXXXXXXXXSMV (SEQ ID NO: 592)

- 140 -

L3.3=QSYDRGXXXXXX (SEQ ID NO: 593)

Randomized mutagenesis of all three light chain CDRs (referred to as L3.1, L3.2, and L3.3) of clone 103-14 was performed. The heavy chain CDR3 (referred to as H3) of clone 103-14 was not mutated. Four randomized libraries based on clone 103-14 (H3 and L3.1, L3.2 & L3.3) were constructed and subjected to a large variety of selection conditions that involved using limiting antigen concentration and the presence or absence of excess free antigen (p40 and p70). The outputs from selections (clones 73-B1 through 99-G11) were screened primarily by BIAcore, and on occasion with RBA and are shown in Table 2 (see Appendix A).

Random mutagenesis of the light chain CDR of 103-14 generated clone Y61, which exhibited a significant improvement in IC_{50} value compared to the parent clone 103-14. Y61 was selected for conversion to a whole IgG1. Whole Y61-IgG1 has an IC_{50} value of approximately 130 pM determined by the PHA assay. The IC_{50} value was not affected by a 50 fold molar excess of free p40, demonstrating that free p40 did not cross-react with Y61 anti-IL-12 antibody to thereby decrease the antibody binding to the heterodimer. The full length sequences of Y61 heavy chain variable region and light chain variable region are shown below.

Y61 Heavy Chain Variable Region Peptide Sequence

CDR H1

QVQLVESGGGVVQPGRSLRLSCAASFTFS **SYGMH** WVRQAPGKGLEWVA

CDR H2

FIRYDGSNKYYADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCKT

CDR H3

HGSHDN WGQGTMTVTSS (SEQ ID NO: 23)

Y61 Light Chain Variable Region Peptide Sequence

- 141 -

CDR L1QSVLTQPPSVSGAPGQRVTISC **SGGRSNIGSNTVK** WYQQLPGTAPKLLIY**CDR L2**5 **GNDQRPS** GVPDRFSGSKSGTSASLAITGLQAEDEADYYC**CDR L3****QSYDRGTHPALL** FGTGTKVTVLG (SEQ ID NO:24)

10 CDR residues are assigned according to the Kabat definitions.

EXAMPLE 2: Mutation of Y61 at Hypermutation and Contact Positions

Typically selection of recombinant antibodies with improved affinities can be
15 carried out using phage display methods. This is accomplished by randomly mutating
combinations of CDR residues to generate large libraries containing single-chain
antibodies of different sequences. Typically, antibodies with improved affinities are
selected based on their ability to reach an equilibrium in an antibody-antigen reaction.
However, when Y61 scFV was expressed on phage surface and incubated with IL-12,
20 selection conditions could not be found that would allow the system to reach normal
antibody-antigen equilibrium. The scFV-phage remained bound to IL-12, presumably
due to a non-specific interaction, since purified Y61 scFv exhibits normal dissociation
kinetics. Since the usual methods of phage-display affinity maturation to Y61 (i.e.
library generation and selections by mutagenesis of multiple CDR residues) could not be
25 utilized, a new strategy was developed in which individual CDR positions were mutated.

This strategy involves selection of appropriate CDR positions for mutation and is
based on identification and selection of amino acids that are preferred selective
mutagenesis positions, contact positions, and/or hypermutation positions. Contact
positions are defined as residues that have a high probability of contact with an antigen
30 when the antigen interacts with the antibody, while hypermutation positions are defined
as residues considered to have a high probability for somatic hypermutation during *in*
vivo affinity maturation of the antibody. Preferred selective mutagenesis positions are
CDR positions that are both contact and hypermutation positions. The Y61 antibody

- 142 -

was already optimized in the CDR3 regions using the procedure described in Example 1, therefore it was difficult to further improve the area which lies at the center of the antibody binding site using phage-display selection methods. Greater improvements in activity were obtained by mutation of potential contact positions outside the CDR3 regions by either removing a detrimental antigen-antibody contact or, engineering a new contact.

Amino acids residues of Y61 which were considered contact points with antigen, and those CDR positions which are sites of somatic hypermutations during *in vivo* affinity maturation, are shown in Table 3 (see Appendix A). For Y61 affinity maturation, 15 residues outside CDR3, 3 residues within the L3 loop, and 5 residues in the H3 loop were selected for PCR mutagenesis.

Y61 scFv gene was cloned into the pUC119(Sfi) plasmid vector for mutagenesis. Oligonucleotides were designed and synthesized with randomized codons to mutate each selected position. Following PCR mutagenesis, a small number of clones (~24) were sequenced and expressed in a host cell, for example, in a bacterial, yeast or mammalian host cell. The expressed antibody was purified and the k_{off} measured using the BIAcore system. Clones with improved off-rates, as compared to Y61, were then tested in neutralization assays. This procedure was repeated for other CDR positions. Individual mutations shown to have improved neutralization activity were combined to generate an antibody with even greater neutralization potency.

The Y61 CDR positions that were mutated in order to improve neutralization potency, and the respective amino-acid substitutions at each position are shown in Figures 2A-2H. Off-rates, as determined by BIAcore analysis, are given. These off rates are also shown in the histograms to the right of each table.

Results of these substitutions at positions H30, H32, H33, H50, H53, H54, H58, H95, H97, H101, L50, L92, L93, demonstrated that all amino-acid substitutions examined resulted in antibodies with poorer off-rates than Y61. At positions H52, L32, and L50, only a one amino acid substitution was found to improve the off-rate of Y61, all other changes adversely affected activity. For L50, this single Gly→Tyr change significantly (5-10 times) improved the neutralization potency of Y61. The results demonstrated the importance of these positions to Y61 activity, and suggest that in most cases phage-display was able to select for the optimal residues. However, at positions

- 143 -

H31, H56, L30, and L94, several substitutions were found to improve Y61 off-rate, suggesting that these positions were also important for antigen binding, although the phage display approach did not allow selection of the optimal residues.

Selective mutation of contact and hypermutation positions of Y61 identified amino acid residue L50 in the light chain CDR2, and residue L94 of the light chain CDR3, which improved the neutralization ability of Y61. A combination of these mutations produced an additive effect, generating an antibody, J695, that exhibited a significant increase in neutralization ability. The full length sequence of J695 heavy and light chain variable region sequences is shown below.

10

J695 Heavy Chain Variable Region Peptide Sequence

CDR H1

QVQLVESGGGVVQPGRSLRLSCAASGFTFS **SYGMH** WVRQAPGKGLEWVA

15

CDR H2

FIRYDGSNKYYADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCKT

CDR H3

20 **HGSHDN** WGQGTMTVTSS (SEQ ID NO: 31)

J695 Light Chain Variable Region Peptide Sequence

25

CDR L1

QSVLTQPPSVSGAPGQRVTISC **SGSRSNIGSNTVK** WYQQLPGTAPKLLIY

CDR L2

YNDQRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC

30

CDR L3

QSYDRYTHPALL FGTGTKVTVLG (SEQ ID NO: 32)

- 144 -

CDR residues are assigned according to the Kabat definitions.

A summary of the heavy and light chain variable region sequence alignments showing the lineage development of clones that were on the path from Joe9 to J695 is shown in Figures 1A-1D. The CDRs and residue numbering are according to Kabat.

EXAMPLE 3: Functional Activity of Anti-hIL-12 Antibodies

To examine the functional activity of the human anti-human IL-12 antibodies of the invention, the antibodies were used in several assays that measure the ability of an antibody to inhibit IL-12 activity.

A. Preparation of Human PHA-activated Lymphoblasts

Human peripheral blood mononuclear cells (PBMC) were isolated from a leukopac collected from a healthy donor by Ficoll-Hypaque gradient centrifugation for 45 minutes at 1500 rpm as described in Current Protocols in Immunology, Unit 7.1. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected and washed three times with phosphate-buffered saline (PBS) by centrifugation for 15 minutes at 1500 rpm to remove Ficoll-Paque particles.

The PBMC were then activated to form lymphoblasts as described in Current Protocols in Immunology, Unit 6.16. The washed PBMC were resuspended at $0.5-1 \times 10^6$ cells/ml in RPMI complete medium (RPMI 1640 medium, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin), supplemented with 0.2% (v/v) PHA-P (Difco, Detroit, MI) and cultured for four days at 37°C in a 5% CO₂ atmosphere. After four days, cell cultures were split 1:1 by volume in RPMI complete medium, plus 0.2% (v/v) PHA-P and 50 U/ml recombinant human IL-2. Recombinant human IL-2 was produced by transfection of an expression vector carrying the human IL-2 cDNA into COS cells (see Kaufman *et al.*, (1991) *Nucleic Acids Res.* 19, 4484-4490), and purified as described in PCT/US96/01382. Cell cultures were then incubated for an additional one to three days. PHA blast cells were harvested, washed twice with RPMI complete medium and frozen in 95% FBS, 5% DMSO at 10×10^6 cells/ml.

- 145 -

PHA blast cells to be used for the IL-12 receptor binding assay (see section B) were collected after one day culture in the presence of IL-2, whereas PHA blast cells to be used for the PHA blast proliferation assay (see section C) and the interferon-gamma induction assay (see section D) were collected after three day culture in the presence of
5 IL-2.

B. IL-12 Receptor Binding Assay

The ability of anti-IL-12 antibodies to inhibit binding of radiolabelled IL-12 to IL-12 receptors on PHA blasts were analyzed as follows. Various concentrations of
10 anti-IL-12 antibody were preincubated for 1 hour at 37°C with 50-100 pM ^{125}I -hIL-12 (iodinated hIL-12 was prepared using the Bolton-Hunter labeling method to a specific activity of 20-40mCi/mg from NEN-Dupont) in binding buffer (RPMI 1640, 5% FBS, 25 mM Hepes pH 7.4). PHA blast cells isolated as described above, were washed once and resuspended in binding buffer to a cell density of 2×10^7 cells/ml. PHA blasts (1×10^6
15 cells) were added to the antibody ^{125}I -hIL-12 mixture and incubated for two hours at room temperature. Cell bound radioactivity was separated from free ^{125}I -hIL-12 by centrifugation of the assay mixture for 30 seconds at room temperature, aspiration of the liquid and a wash with 0.1ml binding buffer, followed by centrifugation at 4°C for 4 min at 10,000 x g. The cell pellet was examined for cell bound radioactivity using a gamma
20 counter. Total binding was determined in the absence of antibody and non-specific binding was determined by inclusion of 25 nM unlabeled IL-12 in the assay. Incubations were carried out in duplicate.

In the IL-12 receptor binding assay using the Y61 and J695 human anti-IL-12 antibodies, both antibodies demonstrated a comparable inhibition of IL-12 receptor
25 binding. Y61 inhibited IL-12 receptor binding with an IC_{50} value of approximately $1.6 \times 10^{-11}\text{M}$, while J695 had an IC_{50} value of approximately $1.1 \times 10^{-11}\text{M}$.

- 146 -

C. Human PHA Blast Proliferation Assay

Anti-IL-12 antibodies were evaluated for their ability to inhibit PHA blast proliferation (which proliferation is stimulated by IL-12). Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 230 pg/ml hIL-12 in
5 100 ml RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar, Cambridge, MA). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of 3×10^5 cells/ml. PHA blasts (100 ml, 3×10^4 cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO₂ and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine
10 (Amersham, Arlington Heights, IL). The culture contents were harvested onto glass fiber filters by means of a cell harvester (Tomtec, Orange, CT) and (³H)-Thymidine incorporation into cellular DNA was measured by liquid scintillation counting. All samples were assayed in duplicate.

The results of neutralization in the presence of varying concentrations of p70:p40
15 (i.e. the ratio of IL-12 heterodimer to free p40 subunit) is shown in Table 4 (see Appendix A).

Analysis of the Y61 human anti-IL-12 antibody in the PHA blast proliferation assay demonstrated that the antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately 1.8×10^{-10} M in the presence of IL-12 p70 alone, without any
20 excess p40 (p70:p40 ratio of 1:0). In the presence of a 50-fold excess of free p40 (p70:p40 at a ratio of 1:50), the Y61 antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately 1.8×10^{-10} M. This result demonstrates that the ability of Y61 to inhibit blast proliferation is not compromised by the presence of excess p40.

The human anti-IL-12 antibody, J695 inhibited PHA blast proliferation with an
25 IC₅₀ value of approximately 1.0×10^{-11} M in the presence of p70:p40 at a ratio of 1:0. In the presence of a p70:p40 ratio of 1:50, this antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately $5.8 \pm 2.8 \times 10^{-12}$ M (n=2), demonstrating that the excess p40 had only a slight inhibitory effect on the antibody. Overall results demonstrate the improved neutralization activity of J695 in comparison with Y61 due to
30 the mutations at L50 and L94.

- 147 -

D. Interferon-gamma Induction Assay

The ability of anti-IL-12 antibodies to inhibit the production of IFN γ by PHA blasts (which production is stimulated by IL-12) was analyzed as follows. Various concentrations of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 200-400 pg/ml hIL-12 in 100 ml RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of 1×10^7 cells/ml. PHA blasts (100 μ l of 1×10^6 cells) were added to the antibody/hIL-12 mixture and incubated for 18 hours at 37°C and 5% CO₂. After incubation, 150 μ l of cell free supernatant was withdrawn from each well and the level of human IFN γ produced was measured by ELISA (Endogen Interferon gamma ELISA, Endogen, Cambridge, MA). Each supernatant was assayed in duplicate.

Analysis of human anti-hIL-12 antibody, Y61 in this assay demonstrated that Y61 inhibited human IFN γ production with an IC₅₀ value of approximately 1.6×10^{-10} M, while the human anti-IL-12 antibody, J695, inhibited human IFN γ production with an IC₅₀ value of approximately $5.0 \pm 2.3 \times 10^{-12}$ M (n=3). The result demonstrates the substantial improvement in the affinity of J695 as a result of the modifications at L50 and L94.

E. Induction of Non-human IL-12 from Isolated PBMC

To examine the cross-reactivity of the human anti-hIL-12 antibodies with IL-12 from other species, non-human IL-12 was produced as follows. PBMC were separated from fresh heparinized blood by density gradient centrifugation as described above using lymphoprep (Nycomed, Oslo, Norway) for cynomolgus monkey, baboon, and dog, PBMC, Accu-paque (Accurate Chemical & Sci. Corp., Westbury, NY) for dog PBMC or Lympholyte-rat (Accurate Chemical & Sci. Corp., Westbury, NY) for rat PBMC.

The PBMC were then induced to produce IL-12 as described (D'Andrea *et al.*, (1992) *J. Exp. Med.* 176, 1387-1398, Villinger *et al.*, (1995) *J. Immunol.* 155, 3946-3954, Buettner *et al.*, (1998) *Cytokine* 10, 241-248). The washed PBMC were resuspended at 1×10^6 cells/ml in RPMI complete medium, supplemented with 0.0075% (wt/vol) of SAC (Pansorbin; Calbiochem-Behring Co., La Jolla, CA) or 1-5 mg/ml

- 148 -

ConA (Sigma Chemical Co., St. Louis, MO) plus 0.0075% SAC and incubated for 18 hours at 37°C in a 5% CO₂ atmosphere. Cell-free and SAC-free medium was collected by centrifugation and filtering through 0.2 mm filters.

IL-12 from the rhesus monkey was obtained as recombinant rhesus IL-12 from
5 Emory University School of Medicine, Atlanta, GA.

F. Murine 2D6 Cell Proliferation Assay

The murine T cell clone 2D6 proliferates in response to murine IL-2, IL-4, IL-7 and IL-12 (Maruo *et al.*, (1997) *J. Leukocyte Biol.* 61, 346-352). A significant
10 proliferation was also detected in response to rat PBMC supernatants containing rat IL-12. The cells do not respond to dog, cynomolgus, baboon or human IL-12. Murine 2D6 cells were propagated in RPMI complete medium supplemented with 50 mM beta-mercaptoethanol (β ME) and 30 ng/ml murine IL-12. One day prior to the assay, the murine IL-12 was washed out and the cells were incubated overnight in RPMI complete
15 medium plus β ME.

Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 40 pg/ml murine IL-12 in 100 ml RPMI complete medium plus β ME in a microtiter plate (U-bottom, 96-well, Costar). 2D6 cells were washed once and resuspended in RPMI complete medium containing β ME to a cell density of 1×10^5
20 cells/ml. 2D6 cells (100 μ l, 1×10^4 cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO₂ and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine. The culture contents were harvested and counted by liquid scintillation counting. All samples were assayed in duplicate.

25 G. Species Cross-reactivity of J695 with Non-Human IL-12

Species cross-reactivity of J695 with non-human IL-12 was analyzed using PBMC's isolated from several non-human species. The presence of non-human IL-12 activity in the rat, dog, cynomolgus and baboon PBMC supernatants was confirmed using several bioassays described above, such as the murine 2D6 cell proliferation assay,
30 the human PHA blast proliferation assay and the interferon-gamma induction assay by blocking the non-human PBMC induced responses with rabbit and/or sheep polyclonal

- 149 -

antibodies to murine and/or human IL-12. Cross-reactivity of the human anti-hIL-12 antibodies Y61 and J695 with non-human IL-12 in PBMC supernatants or purified murine and rhesus IL-12 was then assessed in the same bioassay(s) by determining the J695 antibody concentration at which 50% inhibition of the response was observed. The species cross-reactivity results are summarized in Table 5. The results demonstrate that Y61 and J695 are each able to recognize IL-12 from monkeys (e.g, cynomolgus and rhesus IL-12 for Y61, and cynomolgus, rhesus and baboon for J695) and that J695 is approximately 35 fold less active on dog IL-12; neither Y61 nor J695 cross reacts with mouse or rat IL-12.

H. Human cytokine specificity of J695

The specificity of J695 was tested in a competition ELISA in which a panel of human cytokines was tested for their ability to interfere with the binding of soluble J695 to immobilized human IL-12. The panel of human cytokines included IL-1 α and IL-1 β (Genzyme, Boston, MA), IL-2 (Endogen), IL-4, IL-10, IL-17, IFN-gamma, and TGF- β 1 (R&D, Minneapolis, MN) IL-8 (Calbiochem), PDGF, IGF-I, and IGF-II (Boehringer Mannheim Corp., Indianapolis, IN), TNF α and lymphotoxin, IL-6, soluble IL-6 receptor, IL-11, IL-12 p70, IL-12 p40, M-CSF, and LIF. EBI-3, an IL-12 p40 related protein that is induced by Epstein-Barr virus infection in B lymphocytes (Devergne *et*

Table 5 Species Cross Reactivity Data

Antibody		IC ₅₀ (M)						
Name	Specificity	Mouse IL-12	Rat IL-12	Dog IL-12	Cyno IL-12	Rhesus IL-12	Baboon IL-12	Human IL-1 ^r
		Purified	PBMC sup	PBMC sup	PBMC sup	Purified	PBMC sup	Purified
C17.15	rat- α mulL12	3.0×10^{-11}						
R03B03	rabbit- α mulL12	1.5×10^{-10}	6.0×10^{-10}					
C8.6.2	mouse- α huIL12				1.2×10^{-10}	1.0×10^{-10}	2.0×10^{-10}	5.0×10^{-11}
Y61	human- α huIL12	Non-neutralizing			2.2×10^{-10}	1.0×10^{-10}		1.7×10^{-10}
J695	human- α huIL12	Non-neutralizing	Non-neutralizing	3.5×10^{-10}	1.0×10^{-11}	1.0×10^{-11}	1.5×10^{-11}	5.0×10^{-12}

- 151 -

al., (1996) *J. Virol.* 70, 1143-1153) was expressed as a human IgG-Fc chimera (EBI-3/Fc). Single-stranded salmon sperm DNA (Sigma) was also tested.

Flat-bottom ELISA immunoassay microtiter plates (96 well, high binding, Costar) were coated overnight at 4°C with 0.1 ml human IL-12 (2 µg/ml in 0.1 M carbonate coating buffer (4 volumes 0.1 M NaHCO₃ plus 8.5 volumes 0.1 M NaHCO₃)). The plates were washed twice with PBS containing 0.05 % Tween 20 (PBS-T), blocked with 200 µl of 1 mg/ml bovine serum albumin (BSA, Sigma) in PBS-T for 1 hour at room temperature, and again washed twice with PBS-T. Samples (100 µl) containing IL-12 antibody J695 (100 ng/ml) and each cytokine (2nM) in PBS-T containing 50 µg/ml BSA (PBS-T/BSA) were added and incubated for 2 h at room temperature. The plates were washed 4 times and incubated for 1h at room temperature with 100 µl mouse anti-human lambda-HRP (1:500 in PBS-T/BSA, Southern Biotech. Ass. Inc., Birmingham, AL). The plates were washed 4 times and developed with ABTS (Kirkegaard & Perry Lab., Gaithersburg, MD) for 20-30 minutes in the dark. The OD_{450nm} was read using a microplate reader (Molecular Devices, Menlo Park, CA). Percent binding was determined relative to J695 binding to the IL-12 coated plate in the absence of any soluble cytokine.

The results demonstrated that J695 binding to immobilized human IL-12 was blocked only by human IL-12 p70 and to a lesser extent, by human IL-12 p40 and not by any of the other cytokines tested.

I. Binding to a Novel IL-12 Molecule

An alternative IL-12 heterodimer has been described, in which the p35 subunit is replaced by a novel p19 molecule. P19 was identified using 3D homology searching for IL-6/IL-12 family members, and is synthesized by activated dendritic cells. P19 binds to p40 to form a p19/p40 dimer, which has IL-12 –like activity, but is not as potent as the p35/p40 heterodimer in IFNγ induction. Antibodies which recognize p40 alone, but preferably in the context of a p70 molecule (e.g., J695 and Y61, see Example 3H) are expected to also neutralize both the p35/p40 molecules and the p19/p40 molecules.

EXAMPLE 4: In vivo Activity of Anti-hIL-12 Antibodies

The *in vivo* effects of IL-12 antibodies on IL-12 induced responses were examined in a model modified from one used by Bree *et al.* to study the effect of human IL-12 on peripheral hematology in cynomolgus monkey Bree *et al.*, (1994) *Biochem Biophys Res. Comm.* 204: 1150-1157. In those previous studies, administration of human IL-12 at 1 µg/kg/day for a period of 5 days resulted in a decrease in white blood cell count (WBC), especially in the lymphocyte and monocyte subsets after 24 hours. A decrease in the platelet count was observed at 72 hours. Levels of plasma neopterin, a marker of monocyte activation in response to IFN-γ, began to elevate at 24 hours and were the highest at 72 hours.

In the first study with human anti-hIL-12 antibodies, fifteen healthy cynomolgus monkeys with an average weight of 5kg, were sedated and divided into 5 groups (n=3). Group 1 received an intravenous (IV) administration of 10 mg/kg human intravenous immunoglobulin (IVIG, Miles, Eckhart, IN, purified using protein A Sepharose). Group 2 received an intravenous administration of 1 mg/kg C8.6.2 (neutralizing mouse anti-human IL-12 monoclonal antibody). Group 3 received an intravenous administration of 10 mg/kg C8.6.2. Group 4 received an intravenous administration of 1 mg/kg Y61 (human anti-human IL-12 antibody, purified from CHO cell conditioned medium). Group 5 received an intravenous administration of 10 mg/kg Y61.

One hour after the antibody administration all animals received a single subcutaneous (SC) injection of human IL-12 (1 µg/kg). Blood samples were taken at the following time points: baseline, 8, 24, 48, 96 and 216 hours, and analyzed for complete blood cell counts with differentials and serum chemistry. Serum human IL-12, C8.6.2 antibody, Y61 antibody, monkey IFN-gamma, monkey IL-10, monkey IL-6 and plasma neopterin levels were also measured.

Animals treated with IL-12 plus IVIG control antibody (Group 1) showed many of the expected hematological changes, including decreases in WBC, platelets, lymphocyte count and monocyte count. These decreases were not seen or were less pronounced in the animals treated with either the C8.6.2 or Y61 antibody at 1 or 10 mg/kg (Groups 2-5).

- 153 -

Serum or plasma samples were analyzed by ELISA specific for monkey IFN-gamma and monkey IL-10 (Biosource International, Camarillo, CA), monkey IL-6 (Endogen) and plasma neopterin (ICN Pharmaceuticals, Orangeburg, NY). IFN-gamma, IL-10 or IL-6 were not detected in any of the IL-12 treated animals including the control animals treated with IL-12 plus IVIG. This was probably due to the low level exposure to IL-12 (only 1 dose of 1 µg/kg). Nevertheless, plasma neopterin levels increased about three fold in the IL-12 plus IVIG treated animals but did not change in all C8.6.2 or Y61 treated animals, including the lower dose (1 mg/kg) Y61 treated animals, indicating that Y61 was effective *in vivo* in blocking this sensitive response to IL-12.

In a second study, *in vivo* activity and pharmacodynamics (PD) of J695 in cynomolgous monkeys were studied by administering exogenous rhIL-12 and determining if J695 could block or reduce the responses normally associated with rhIL-12 administration. Male cynomolgus monkeys (n=3 per group) were administered a single dose of 0.05, 0.2, or 1.0 mg/kg J695 or 1 mg/kg intravenous immunoglobulin (IVIG) as a bolus intravenous (IV) injection via a saphenous vein or subcutaneously (SC) in the dorsal skin. One hour following the administration of J695 or IVIG, all animals received a single SC dose of 1 µg/kg rhIL-12 in the dorsal skin. Blood samples were collected via the femoral vein up to 28 days after J695 administration. Serum was acquired from each blood sample and assayed for IL-12, J695, IFN-γ, and anti-J695 antibodies by ELISA. Neopterin was assayed by reverse-phase high performance liquid chromatography.

The levels of neopterin, normalized with respect to the levels of neopterin that were measured before administration of J695 or rhIL-12, are shown in Figure 3. To compare the suppression of neopterin between groups, the area under the curve (AUC) normalized for neopterin levels was calculated for each animal (Table 6). Neopterin exposure (AUC) was suppressed in a dose-dependent manner between approximately 71 and 93% in the IV groups and between 71 and 100% in SC groups, relative to the IVIG control groups. These results suggest that the dose of J695 necessary for 50% inhibition of the neopterin response (ED₅₀) was less than 0.05 mg/kg when administered by either the IV or SC route.

Table 6: Dose-Dependent Suppression of IL-12 Induced Neopterin by J695 in Cynomolgus Monkeys

Route of dosing IVIG or J695 and rhIL-12	J695 Dose (mg/kg)	IVIG Dose (mg/kg)	AUC of Normalized Neopterin Levels	% Reduction of Neopterin AUC Compared with Control
Single IV injection followed 1 hr later by a dose of 1 µg/kg human IL-12 given SC	-	1.0	1745 ± 845	0
	0.05	-	502 ± 135	71.3
	0.2	-	199 ± 316	88.6
	1.0	-	128 ± 292	92.7
Single SC injection followed 1 hour later by a dose of 1 µg/kg human IL-12 given SC	-	1.0	1480 ± 604	0
	0.05	-	426 ± 108	71.2
	0.2	-	395 ± 45.9	73.3
	1.0	-	0 ± 109	100

5 Treatment with J695 also prevented or reduced the changes in hematology normally associated with rhIL-12 administration (leukopenia and thrombocytopenia). At 24 hours after rhIL-12 administration lymphocyte counts were reduced by approximately 50% when compared to baseline values in the control IV and SC IVIG treated groups. Administration of J695 either SC or IV at all three dose levels prevented
 10 this reduction, resulting in lymphocyte counts at 24 hours approximately the same as baseline values. At 48 hours after IL-12 administration, platelet counts in the groups treated with IV and SC IVIG were reduced by approximately 25% when compared to baseline values.

An example dose schedule targeted to maintain serum levels above the 90%
 15 effect level would be 1 mg/kg IV and SC given approximately every other week, or 0.3 mg/kg given approximately every week, assuming slight accumulation during repeated dosing. This study demonstrates that antibody can be given safely to monkeys at such dosages. In independent toxicity studies, it was further found that up to 100 mg/kg of the antibody can be given safely to monkeys.

20 J695 was also effective in preventing IFN-γ production in mice treated with a chimeric IL-12, a molecule which combines the murine p35 subunit with the human IL-12 p40 subunit. In contrast to human IL-12 which is biologically inactive in mice, this

- 155 -

chimeric IL-12 retains biological function in mice, including induction of IFN- γ . In addition, the human p40 subunit allows the molecule to be bound and neutralized by J695. Chimeric IL-12 at a dose of 0.05 mg/kg i.p. was administered to female C3H/HeJ mice (10/experimental group) in five daily doses on days 0, 1, 2, 3, and 4. J695 was
5 given on days 0, 2 and 4 at doses of 0.05, 0.01, 0.002, 0.0004, 0.00008, and 0.000016 mg/kg i.p., 30' prior to the IL-12 injections. The control hulgG1 γ was given IP. at a dose of 0.05 mg/kg on days 0, 2, and 4. The mice were bled on day 5, and serum IFN- γ levels were determined by ELISA. The results demonstrated that J695 caused dose-dependent inhibition of IFN- γ production with an ED₅₀ of approximately 0.001 mg/kg.
10 Collectively, these results demonstrate that J695 is a potent inhibitor of IL-12 activity *in vivo*.

**EXAMPLE 5: Kinetic Analysis of Binding of Human Antibodies to
Recombinant human IL-12 (rhIL-12)**

15

Real-time binding interactions between captured ligand (human anti-rhIL-12 antibody J695, captured on a biosensor matrix) and analyte (rhIL12 in solution) were measured by surface plasmon resonance (SPR) using the BIAcore system (Biacore AB, Uppsala, Sweden). The system utilizes the optical properties of SPR to detect
20 alterations in protein concentration within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units
25 (RU) and are displayed with respect to time along the y-axis of a sensorgram.

To facilitate immobilization of goat anti-human IgG (Southern Biotechnology Associates, Cat. No. 2040-01, Birmingham, AL) on the biosensor matrix, goat anti-human IgG is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100 mM N-hydroxysuccinimide (NHS)
30 and 400 mM N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Next, goat anti-human IgG is injected across the activated matrix. Thirty-five microliters of goat anti-human IgG (25 μ g/ml), diluted in sodium acetate, pH 4.5, is

injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. Standard amine coupling kits were commercially available (Biacore AB, Cat. No. BR-1000-50, Uppsala, Sweden).

5 J695 was diluted in HBS running buffer (Biacore AB, Cat. No. BR-1001-88, Uppsala, Sweden) to be captured on the matrix via goat anti-human IgG. To determine the capacity of rhIL12-specific antibodies to bind immobilized goat anti-human IgG, a binding assay was conducted as follows. Aliquots of J695 (25 µg/ml; 25 µl aliquots) were injected through the goat anti-human IgG polyclonal antibody coupled dextran
10 matrix at a flow rate of 5 µl/min. Before injection of the protein and immediately afterward, HBS buffer alone flowed through each flow cell. The net difference in signal between the baseline and the point corresponding to approximately 30 seconds after completion of J695 injection was taken to represent the amount of IgG1 J695 bound (approximately 1200 RU's). Direct rhIL12 specific antibody binding to soluble rhIL12
15 was measured. Cytokines were diluted in HBS running buffer and 50 µl aliquots were injected through the immobilized protein matrices at a flow rate of 5 µl/min. The concentrations of rhIL-12 employed were 10, 20, 25, 40, 50, 80, 100, 150 and 200 nM. Prior to injection of rhIL-12, and immediately afterwards, HBS buffer alone flowed through each flow cell. The net difference in baseline signal and signal after completion
20 of cytokine injection was taken to represent the binding value of the particular sample. Biosensor matrices were regenerated using 100 mM HCl before injection of the next sample. To determine the dissociation constant (off-rate), association constant (on-rate), BIAcore kinetic evaluation software (version 2.1) was used.

25 Representative results of CHO derived J695 binding to rhIL-12 as compared to the COS derived J695, are shown in Table 7.

Table 7: Binding of CHO or COS derived J695 to rhIL-12.

Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
CHO	200	1112	1613	1.48
CHO	150	1033	1525	1.45
CHO	100	994	1490	1.43

- 157 -

CHO	80	955	1457	1.40
CHO	50	912	1434	1.36
CHO	40	877	1413	1.33
CHO	25	818	1398	1.25
CHO	20	773	1382	1.20
CHO	10	627	1371	0.98
Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
COS	200	1172	1690	1.49
COS	150	1084	1586	1.46
COS	100	1024	1524	1.44
COS	80	985	1489	1.42
COS	50	932	1457	1.37
COS	40	894	1431	1.34
COS	25	833	1409	1.27
COS	20	783	1394	1.20
COS	10	642	1377	1.00

Molecular kinetic interactions between captured J695 and soluble rhIL-12 were quantitatively analyzed using BIAcore technology. Several independent experiments were performed and the results were analyzed by the available BIAcore mathematical analysis software to derive kinetic rate constants, as shown in Table 8.

Table 8: Apparent kinetic rate and affinity constants of J695 for rhIL-12.

Antibody	Source	On-rate (M ⁻¹ s ⁻¹), Avg.	Off-rate (s ⁻¹), Avg.	Kd (M), Avg.
J695	CHO	3.52E+05	4.72E-05	1.34E-10
J695	COS	3.40E+05	2.61E-05	9.74E-11

There was a small difference between the calculated apparent constant (Kd) for the interaction between CHO derived J695 ($K_d = 1.34 \times 10^{-10} \text{M}^{-1}$) and COS derived J695 ($K_d = 9.74 \times 10^{-11} \text{M}^{-1}$) antibodies. The apparent dissociation constant (Kd) between J695 and rhIL12 was estimated from the observed rate constants by the formula: $K_d = \text{off-rate} / \text{on-rate}$.

- 158 -

To determine the apparent association and dissociation rate constant for the interaction between J695 and rhIL-12, several binding reactions were performed using a fixed amount of J695 (2 µg/ml) and varying concentrations of rhIL-12. Real-time binding interaction sensorgrams between captured J695 and soluble rhIL12 showed that both forms of antibody were very similar for both the association and dissociation phase.

To further evaluate the capacity of captured IgG1 J695 mAb to bind soluble recombinant cytokine, a direct BIAcore method was used. In this method, goat anti-human IgG (25 µg/ml) coupled carboxymethyl dextran sensor surface was coated with IgG1 J695 (2µg/ml) and recombinant cytokine was then added. When soluble rhIL12 was injected across a biosensor surface captured with CHO or COS derived IgG1 J695, the amount of signal increased as the concentration of cytokine in the solution increased. No binding was observed with rmIL12 (R&D Systems, Cat. No. 419-ML, Minneapolis, MN) or rh IL12 any concentration tested up to 1000 nM. These results support the conclusion that IgG1 J695 antibodies recognize a distinct determinant on rhIL-12.

Table 9 shows the results of an experiment using BIAcore to demonstrate human IgG1 J695 mAb binding to only soluble rhIL12 and none of the other recombinant cytokines.

Table 9: Epitope mapping of J695 using BIAcore technology.

	Captured ligand COS J695	Captured ligand CHO J695
Soluble analyte		
rec. human IL12	Positive	Positive
rec. murine IL12	Negative	Negative

EXAMPLE 6: Further Studies of J695 Affinity for IL-12

Molecular kinetic interactions between J695 antibody and human IL-12 were quantitatively analyzed using BIAcore plasmon resonance technology, and apparent kinetic rate constants were derived.

- 159 -

BIAcore technology was used to measure the binding of soluble rhIL-12 to solid phase captured J695. A goat anti-human IgG antibody was immobilized on the biosensor chips, then a fixed amount of J695 was injected and captured on the surface. Varying concentrations of rhIL-12 were applied, and the binding of IL-12 at different concentrations to J695 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming zero-order dissociation and first order association kinetics, as well as a simple one-to-one molecular interaction between J695 and IL-12. Three independent experiments were performed, and the values shown are averages for the three experiments. From these measurements, the apparent dissociation (k_d) and association (k_a) rate constants were derived and used to calculate a K_d value for the interaction (see Table 10). The results indicated that J695 has a high affinity for rhIL-12.

Table 10: Kinetic Parameters for the Interaction Between J695 and Human IL-12

Kinetic Parameter	Value
k_d	$3.71 \pm 0.40 \times 10^{-5} \text{ s}^{-1}$
k_a	$3.81 \pm 0.48 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$
K_d	$9.74 \times 10^{-11} \text{ M}$ (14 ng/mL)

EXAMPLE 7: Characteristics and Neutralization Activity of C17.15, a Rat Monoclonal Antibody to Murine Interleukin-12

To assess the relevance of IL-12 treatment studies in mouse models of inflammation and autoimmunity using monoclonal antibodies specific for murine IL-12 to similar approaches in human disease, the interaction of C17.15, a rat anti-murine IL-12 monoclonal antibody with murine IL-12, was examined. The ability of C17.15 to neutralize murine IL-12 activity in a PHA blast proliferation assay, and to block murine IL-12 binding to cell surface receptors, was assessed, as were the kinetics of the C17.15-murine IL-12 binding interaction.

- 160 -

In a human PHA blast proliferation assay (See Example 3), serial dilutions of C17.15 or rat IgG2a (a control antibody) were preincubated with 230 pg/mL murine IL-12 for 1 hr at 37 °C. PHA-stimulated blast cells were added to the antibody-IL-12 mixtures and incubated for 3 days at 37 °C. The cells were subsequently labeled for 6 h with 1 μ Ci/well [3 H]-thymidine. The cultures were harvested and [3 H]-thymidine incorporation was measured. Background non-specific proliferation was measured in the absence of added murine IL-12. All samples were assayed in duplicate. The IC_{50} (M) of C17.15 for recombinant murine IL-12 in this assay was found to be 1.4×10^{-11} , as compared to the IC_{50} value of 5.8×10^{-12} observed for J695 for recombinant human IL-12 under the same conditions (see Table 11).

Table 11: Comparison of the properties of anti-human IL-12 monoclonal antibody J695 and the rat anti-mouse IL-12 monoclonal antibody C17.15

Antibody	Epitope	Biomolecular Interaction Assay			Receptor Binding Assay	PHA blast Assay
		k_a , on-rate ($M^{-1}s^{-1}$)	k_d , off-rate (s^{-1})	K_d (M)	IC_{50} (M)	IC_{50} (M)
J695	Hu p40	3.81×10^5	3.71×10^{-5}	9.74×10^{-11}	1.1×10^{-11}	5.8×10^{-12}
C17.15	Mu p40	3.80×10^5	1.84×10^{-4}	4.80×10^{-10}	1.5×10^{-10}	1.4×10^{-11}

15

The ability of C17.15 to inhibit the binding of murine IL-12 to cellular receptors was also measured. Serial dilutions of C17.15 were pre-incubated for 1 hr at 37 °C with 100 pM [125 I]-murine IL-12 in binding buffer. The 2D6 cells (2×10^6) were added to the antibody/[125 I]-murine IL-12 mixture and incubated for 2 hours at room temperature. Cell-bound radioactivity was separated from free [125 I]-IL-12, and the remaining cell-bound radioactivity was determined. Total binding of the labeled murine IL-12 to receptors on 2D6 cells was determined in the absence of antibody, and non-specific binding was determined by the inclusion of 25 nM unlabelled murine IL-12 in the assay. Specific binding was calculated as the total binding minus the non-specific binding. Incubations were carried out in duplicate. The results showed that C17.15 has an IC_{50} (M) of 1.5×10^{-10} for inhibition of binding of murine IL-12 to cellular receptors.

25

- 161 -

The affinity of C17.15 for recombinant murine IL-12 was assessed by biomolecular interaction analysis. A goat anti-rat IgG antibody was immobilized on the biosensor chips, followed by an injection of a fixed amount of the C17.15 antibody, resulting in capture of C17.15 on the surface of the chip. Varying concentrations of recombinant murine IL-12 were applied to the C17.15 surface, and the binding of murine IL-12 to the immobilized C17.15 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming a zero order dissociation and first order association kinetics as well as a simple one to one molecular interaction between the immobilized C17.15 and murine IL-12. From these measurements, the apparent dissociation (k_d , off-rate) and association (k_a , on-rate) rate constants were calculated. These results were used to calculate a K_d value for the interaction. An on-rate of $3.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, an off-rate of $1.84 \times 10^{-4} \text{ s}^{-1}$, and a K_d of 4.8×10^{-10} was observed for the recombinant murine IL-12-C17.15 interaction.

The observed activities of C17.15 in neutralizing murine IL-12 activity and binding to cell surface receptors, as well as the kinetics of binding of C17.15 to murine IL-12 correlate with similar measurements for the J695-rhIL-12 interaction. This indicates that the modes of action of the rat anti-mouse IL-12 antibody C17.15 and anti-human IL-12 antibody J695 are nearly identical based upon on-rate, off-rate, K_d , IC_{50} , and the PHA blast assay. Therefore, C17.15 was used as a homologous antibody to J695 in murine models of inflammation and autoimmune disease to study the effects of IL-12 blockade on the initiation or progression of disease in these model animals (see Example 8).

EXAMPLE 8: Treatment of Autoimmune or Inflammation-Based Diseases in Mice by α -Murine IL-12 Antibody Administration

A. Suppression of Collagen-Induced Arthritis in Mice by the α -IL-12 antibody C17.15

A correlation between IL-12 levels and rheumatoid arthritis (RA) has been demonstrated. For example, elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita et al (1998) *Arthritis and Rheumatism*, 41: 306-314). Therefore, the ability of C17.15, a rat anti-mouse IL-12 antibody, to suppress collagen-induced arthritis in mice was assessed.

- 162 -

Male DBA/1 mice (10/group) were immunized with type II collagen on Day 0 and treated with C17.15, or control rat IgG, at 10 mg/kg intraperitoneally on alternate days from Day -1 (1 day prior to collagen immunization) to Day 12. The animals were monitored clinically for the development of arthritis in the paws until Day 90. The arthritis was graded as: 0- normal; 1- arthritis localized to one joint; 2- more than one joint involved but not whole paw; 3- whole paw involved; 4- deformity of paw; 5- ankylosis of involved joints. The arthritis score of a mouse was the sum of the arthritic grades in each individual paw of the mouse (max = 20). The results are expressed as mean \pm SEM in each group.

The results, as shown in Figure 4, indicate that an arthritic score was measurable in the C17.15-treated mice only after day 50 post-treatment, and that the peak mean arthritic score obtained with the C17.15-treated mice was at least 5-fold lower than that measured in the IgG-treated mice. This demonstrated that the rat anti-mouse IL-12 antibody C17.15 prevented the development of collagen-induced arthritis in mice.

B. Suppression of Colitis in Mice by the Rat α -Murine IL-12 Antibody C17.15

IL-12 has also been demonstrated to play a role in the development/pathology of colitis. For example, anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, e.g., TNBS induced colitis IL-2 knockout mice (Simpson et al. (1998) *J. Exp. Med.* 187(8): 1225-34). Similarly, anti-IL-2 antibodies have been demonstrated to suppress colitis formation in IL-10 knock-out mice. The ability of the rat anti-mouse IL-12 antibody, C17.15, to suppress TNBS colitis in mice was assessed in two studies (Davidson et al. (1998) *J. Immunol.* 161(6): 3143-9).

In the first study, colitis was induced in pathogen free SJL mice by the administration of a 150 μ L 50% ethanol solution containing 2.0 mg TNBS delivered via a pediatric umbilical artery catheter into the rectum. Control animals were treated with a 150 μ L 50% ethanol solution only. A single dose of 0.75, 0.5, 0.25, or 0.1 mg C17.15 or 0.75 mg control rat IgG2a was given intravenously via the tail vein at day 11, and the therapeutic effect of the treatment was assessed by weighing the animals on days 11 and 17, and histological scoring at day 17. The weight of the mice treated with C17.15 increased within 48 hours of antibody treatment and normalized on day 6 after treatment. The effect of treatment with C17.15 was confirmed histologically. Further,

- 163 -

assessments of IFN- γ secretion by CD4⁺ T-cells from spleen and colon of the treated mice, as well as IL-12 levels from spleen or colon-derived macrophages from the treated mice were also made (see Table 12).

In the second study, the dosing was optimized and the mice were treated with a total dose of 0.1 mg or 0.5 mg C17.15 or 0.1 mg control IgG2a, respectively, split between days 12 and 14. It was found that the administration of C17.15 in a single dose at the dosage of 0.1 mg/mouse or 0.25 mg/mouse led to only partial improvement in TNBS-induced colitis and did not result in a significant reduction in the CD4⁺ T cell production of IFN- γ *in vitro*, but did result in a significant decrease in secretion of IL-12, compared to untreated controls. At a single dose of 0.5 mg/mouse or greater a response was observed. Taking the lowest dose of antibody tested and administering it in two divided injections (at days 12 and 14) improved the dosing regimen, indicating that multiple low doses can be more effective than a single bolus dose. The data obtained are shown in Table 12.

Table 12: Anti-mouse IL-12 mAb C17.15 Suppresses Established Colitis in Mice

Disease Induction Day 0	Treatment Day 11	Weight (g)		IFN- γ spleen CD4 ⁺ cells (U/mL)	IL-12 spleen macrophages (pg/ml)
		Day 11	Day 17		
TNBS + Ethanol	Control IgG2a 0.75 mg	16.0	15.26	3326	300
TNBS + Ethanol	C17.15 0.75 mg	16.0	20.21	1732	0
TNBS + Ethanol	C17.15 0.5 mg	16.36	19.94	1723	0
TNBS + Ethanol	C17.15 0.25 mg	16.28	17.7	3618	7
TNBS + Ethanol	C17.15 0.1 mg	16.2	17.98	3489	22
Ethanol control	-	20.76	21.16	1135	0

Administration of C17.15 monoclonal anti-IL-12 in two divided doses spaced one day apart totaling 0.1 mg/mouse or 0.05 mg/mouse led to complete reversal of colitis as assessed by wasting and macroscopic appearance of the colon. In addition, this dose schedule led to significant down-regulation of lamina propria T-cell production of IFN- γ and macrophage production of IL-12, so that the latter were comparable to

levels seen in control ethanol-treated mice without TNBS-colitis. Thus, C17.15 administration to mouse models for TNBS colitis reversed the progression of the disease in a dose-dependent manner.

C. Suppression of Experimental Autoimmune Encephalomyelitis (EAE) in Mice by α -

5 IL-12 Antibodies

It is commonly believed that IL-12 plays a role in the pathogenesis of multiple sclerosis (MS). The inducible IL-12 p40 message has been shown to be expressed in acute plaques of MS patients but not in inflammatory brain infarct lesions (Windhagen, A. *et al.* (1995) *J. Exp. Med.* 182: 1985-1996). T cells from MS patients (but not control
10 T cells) stimulate IL-12 production from antigen-presenting cells through unregulated CD40L expression (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). MS patients have enhanced IFN- γ secretion that can be blocked with α -IL-12 antibodies *in vitro* (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). Elevated levels of serum IL-12 are detected in MS patients, but not in other
15 neurological diseases (Nicoletti, F. *et al.* (1996) *J. Neuroimmunol.* 70: 87-90). Increased IL-12 production has been shown to correlate with disease activity in MS patients (Cormabella, M. *et al.* (1998) *J. Clin. Invest.* 102: 671-678). The role of IL-12 in the pathogenesis of a murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), has been studied (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:
20 281-386; Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33; and Segal, B.M. *et al.* (1998) *J. Exp. Med.* 187: 537-546). The disease in this model is known to be induced by T cells of the TH₁ subset. Therefore, the ability of α -IL-12 antibodies to prevent the onset of acute EAE was assessed.

An α -IL-12 antibody was found to be able to inhibit the onset of acute EAE, to
25 suppress the disease after onset, and to decrease the severity of relapses in mice immunized with the autoantigen, myelin basic protein (Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33). The beneficial effects of α -IL-12 antibody treatment in the mice persisted for over two months after stopping treatment. It has also been demonstrated that anti-IL-12 antibodies suppress the disease in mice that are recipients
30 of encephalitogenic T cells by adoptive transfer (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181: 281-386).

EXAMPLE 9: Clinical Pharmacology of J695

In a double blind, crossover study, 64 healthy, human male subjects were administered ascending doses of J695 or placebo. Measurement of complement
5 fragment C3a prior to and 0.25 h after dosing did not demonstrate activation of the complement system. CRP and fibrinogen levels were only increased in subjects in whom symptoms of concurrent infections were observed.

All subjects survived and the overall tolerability of J695 was very good. In no case did treatment have to be stopped because of adverse events (AEs). The most
10 commonly observed AEs were headache and common cold/bronchitis, neither of which were categorized as severe.

One of the study subjects, a 33-year-old single male, was suffering from psoriasis guttata at the start of the study. According to the randomized study design, this subject by chance received 5mg/kg J695 by SC administration. Ten days prior to
15 administration of the antibody, the subject showed only small discrete papular lesions on the arms and legs. At the time of the antibody administration, the subject displayed increased reddening, thickness of the erythematous plaques, and increased hyperkeratosis. One week after J695 administration, the subject reported an improvement in skin condition, including flattening of the lesions and a decrease in
20 scaling. Shortly after the second administration of J695 (5 mg/kg IV), the subject's skin was totally cleared of psoriatic lesions, in the absence of any local treatment. Erythematous plaques covered with white scales reappeared concomitant with the expected clearance of J695 after the second administration of antibody.

25 EXAMPLE 10: Comparison of J695 Produced by Two CHO Cell Lines

For recombinant expression of J695, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells (Urlaub, G. and Chasin, L.A. (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220)
30 by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and

- 166 -

the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate
5 selection/amplification.

One hundred and fifty micrograms of an expression vector encoding the peptide sequences of the human antibody J695 were dissolved in 2.7 ml water in a 50 ml conical tube. Three hundred μ L of 2.5 M CaCl_2 were added and this DNA mixture was added dropwise to 3 ml of 2 x HEPES buffered saline in a 50 ml conical tube. After
10 vortexing for 5 sec and incubating at room temperature for 20 min, 1 mL was distributed evenly over each plate (still in F12 medium), and the plates were incubated at 37 °C for 4 h. Liquid was removed by aspiration and 2 ml of 10% DMSO in F12 were added to each plate. The DMSO shock continued for 1 min, after which the DMSO was diluted by the addition of 5 ml PBS to each plate. Plates were washed twice in PBS, followed
15 by the addition of 10 ml of alpha MEM, supplemented with H/T and 5% FBS (selective for cells expressing DHFR) and overnight incubation at 37 °C. Cells were seeded into 96-well plates at a density of 100 cells per well, and plates were incubated at 37 °C, 5% CO_2 for two weeks, with one change of medium per week.

Five days after the final medium change, culture supernatants were diluted 1:50
20 and tested using an ELISA specific for human IgG gamma chain. The clones yielding the highest ELISA signal were transferred from the 96-well plates to 12-well plates in 1.5 ml/well of Alpha MEM + 5% dialyzed serum. After 3 days, another ELISA specific for human IgG gamma chain was performed, and the 12 clones with the greatest activity were split into the alpha MEM + 5% dialyzed serum and 20 nM MTX. Cell line 031898
25 218 grew in the presence of 20 nM MTX without any apparent cell death or reduction in growth rate, produced 1.8 μ g/ml hIgG in a three-day assay. T-25 cultures of 031898 218, growing in medium containing MTX, produced an average of 11.9 μ g/ml of J695. The line, designated ALP903, was adapted to growth in suspension under serum-free conditions, where it produced 7.5 pg J695/cell/24h.

30 ALP903 cells, after initial selection in alpha MEM/5% FBS/20 nM MTX medium, were passed again in 20 nM MTX. The cells were cultured under 100 nM MTX selection, followed by passaging in 500 nM MTX twice in the next 30 days. At

- 167 -

that time the culture was producing 32 μg J695/mL/24 h. The culture was subcloned by limiting dilution. Subclone 218-22 produced 16.5 μg /mL in a 96-well plate in 2 days and 50.3 μg /mL of J695 in a 12-well dish in 2 days. Clone 218-22 was cultured in alpha MEM/5% dialyzed FBS/500 nM MTX for 38 days, followed by adaptation to serum-free spinner culture, as above. The average cell-specific productivity of the serum-free suspension culture, designated ALP 905, was 58 pg/cell/24h.

The first cell line used to produce J695 (ALP 903) resulted in lower yields of the antibody from culture than a second cell line, ALP 905. To assure that the ALP 905-produced J695 was functionally identical to that produced from ALP 903, both batches of antibodies were assessed for IL-12 affinity, for the ability to block IL-12 binding to cellular receptors, for the ability to inhibit IFN- γ induction by IL-12, and for the ability to inhibit IL-12-mediated PHA blast proliferation.

The affinities of J695 batches ALP 903 and ALP 905 for IL-12 were determined by measuring the kinetic rate constants of binding to IL-12 by surface plasmon resonance studies (BIAcore analyses). The off-rate constant (k_d) and the on-rate constant (k_a) of antibody batches ALP903 and ALP905 for binding to rhIL-12 were determined in three experiments (as described in Example 3). The affinity, K_d , of binding to IL-12 was calculated by dividing the off-rate constant by the on-rate constant. K_d was calculated for each separate experiment and then averaged. The results showed that the determined kinetic parameters and affinity of binding to rhIL-12 were very similar for J695 batches ALP 903 and ALP 905: the calculated K_d was $1.19 \pm 0.22 \times 10^{-10}$ M for batch ALP 903 and $1.49 \pm 0.47 \times 10^{-10}$ M for batch ALP 905 (see Table 13).

The ability of J695 derived from both ALP 903 and ALP 905 to block binding of rhIL-12 to IL-12 receptors on human PHA-activated T-lymphoblasts was assessed (see Example 3). Each sample of J695 was tested at a starting concentration of 1×10^{-8} with 10-fold serial dilutions. The antibody was preincubated for 1 hour at 37 °C with 50 pM [^{125}I]-human IL-12 in binding buffer. PHA blast cells were added to the antibody/[^{125}I]-human IL-12 mixture and incubated for 2 h at room temperature. Cell bound radioactivity was separated from free [^{125}I]-IL-12 by centrifugation and washing steps, and % inhibition was calculated. The IC_{50} values for J695 were determined from the inhibition curves using 4-parameter curve fitting and were confirmed by two

independent experiments. Incubations were carried out in duplicate. The results for the two batches of J695 were very similar (see Table 13).

The ability of J695 from both ALP 903 and ALP 905 cells to inhibit rhIL-12-induced IFN- γ production by human PHA-activated lymphoblasts *in vitro* was assessed. Serial dilutions of J695 were preincubated with 200 pg/mL rhIL-12 for 1 h at 37 °C. PHA lymphoblast cells were added and incubated for 18 hours at 37 °C. After incubation, cell free supernatant was withdrawn and the level of human IFN- γ determined by ELISA. The IC₅₀ values from the inhibition curves were plotted against the antibody concentration using 4-parameter curve fitting. The results demonstrate that the ability of the two batches to inhibit IFN- γ production is very similar.

The *in vitro* PHA blast cell proliferation assay was used to measure the neutralization capacity of ALP 903 and ALP 905 J695 for rhIL-12. Serial dilutions of J695 of each type were preincubated with 230 pg/mL human IL-12 for 1 h at 37 °C. Next PHA blast cells were added and incubated for 3 days at 37 °C. The cells were then labeled for 6 hours with 1 γ Ci/well [³H]-thymidine. The cultures were harvested and [³H]-thymidine incorporation measured. Non-specific proliferation (background) was measured in the absence of rhIL-12. The IC₅₀ values for ALP 903 and ALP 905 J695 were found to be very similar and are set forth in Table 13.

The activity of the J695 antibodies in neutralizing rhIL-12 activity, in blocking IL-12 binding to cell surface receptors, and in binding to rhIL-12 did not significantly differ from batch ALP 903 to batch ALP 905, and thus the antibodies produced from these two different cell types were equivalent.

Table 13: Comparison of the Properties of J695 lots ALP 903 and ALP 905

Antibody	k _{on} , On-rate (M ⁻¹ , s ⁻¹)	k _d , Off-rate (s ⁻¹)	K _d (M)	RB assay IC ₅₀ (M)	PHA blast Assay IC ₅₀ (M)	IFN- γ Assay IC ₅₀ (M)
J695 ALP 903	3.75 x 10 ⁵	4.46 x 10 ⁻⁵	1.19 x 10 ⁻¹⁰	3.4 x 10 ⁻¹¹	5.5 x 10 ⁻¹²	5.8 x 10 ⁻¹²
J695 ALP 905	3.91 x 10 ⁵	5.59 x 10 ⁻⁵	1.49 x 10 ⁻¹⁰	3.0 x 10 ⁻¹¹	4.4 x 10 ⁻¹²	4.3 x 10 ⁻¹²

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. An isolated human antibody, or an antigen-binding portion thereof, that binds to human IL-12, wherein said human antibody is a neutralizing antibody.

5

2. A selectively mutated human IL-12 antibody, comprising:
a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis, contact or hypermutation position with an activity enhancing amino acid residue such that it binds to human IL-12.

10

3. A selectively mutated human IL-12 antibody, comprising:
a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position with an activity enhancing amino acid residue such that it binds to human IL-12.

15

4. The selectively mutated human IL-12 antibody of claim 2, wherein the human antibody or antigen-binding portion thereof is selectively mutated at more than one preferred selective mutagenesis, contact or hypermutation positions with an activity enhancing amino acid residue.

20

5. The selectively mutated human IL-12 antibody of claim 4, wherein the human antibody or antigen-binding portion thereof is selectively mutated at no more than three preferred selective mutagenesis, contact or hypermutation positions.

25

6. The selectively mutated human IL-12 antibody of claim 4, wherein the human antibody or antigen-binding portion thereof is selectively mutated at no more than two preferred selective mutagenesis, contact or hypermutation positions.

- 171 -

7. The selectively mutated human IL-12 antibody of claim 2, wherein the human antibody or antigen-binding portion thereof, is selectively mutated such that a target specificity affinity level is attained, said target level being improved over that attainable when selecting for an antibody against the same antigen using phage display
5 technology.

8. The selectively mutated human IL-12 antibody of claim 7, wherein the selectively mutated human antibody further retains at least one desirable property or characteristic.

10

9. An isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast
15 proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6}\text{M}$ or less.

10. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-2}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin
20 blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-7}\text{M}$ or less.

11. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-3}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin
25 blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-8}\text{M}$ or less.

12. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-4}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin
30 blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}\text{M}$ or less.

- 172 -

13. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-5} \text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-10} \text{M}$ or less.

5

14. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-5} \text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-11} \text{M}$ or less.

10

15. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-6} \text{M}$ or less;

15 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

20 16. The isolated human antibody of claim 15, or an antigen-binding portion thereof, which further has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4.

25 17. The isolated human antibody of claim 15, or an antigen-binding portion thereof, which further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

- 173 -

18. The isolated human antibody, or antigen binding portion thereof of claim 16, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

5

19. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}M$ or less;

10 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

15 20. The isolated human antibody of claim 19, or an antigen-binding portion thereof, which further has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12.

20 21. The isolated human antibody of claim 19, or an antigen-binding portion thereof, which further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

25 22. The isolated human antibody of claim 19, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

- 174 -

23. An isolated human antibody, or an antigen-binding portion thereof, which
a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay
with an IC₅₀ of 1×10^{-9} M or less;

b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID
5 NO: 17; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID
NO: 18.

24. The isolated human antibody, or an antigen-binding portion thereof, of
10 claim 23 which further has a heavy chain CDR2 comprising the amino acid sequence of
SEQ ID NO: 19; and a light chain CDR2 comprising the amino acid sequence of SEQ
ID NO: 20.

25. The isolated human antibody, or an antigen-binding portion thereof, of
15 claim 23 which further has a heavy chain CDR1 comprising the amino acid sequence of
SEQ ID NO: 21; and a light chain CDR1 comprising the amino acid sequence of SEQ
ID NO: 22.

26. An isolated human antibody, or an antigen-binding portion thereof,
20 having a heavy chain variable region comprising the amino acid sequence of SEQ ID
NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ
ID NO: 24.

27. The isolated human antibody of claim 26, comprising a heavy chain
25 constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA
and IgE constant regions.

28. The isolated human antibody of claim 27, wherein the antibody heavy
chain constant region is IgG1.

30

29. The isolated human antibody of claim 26, which is a Fab fragment.

- 175 -

30. The isolated human antibody of claim 26, which is a F(ab')₂ fragment.

31. The isolated human antibody of claim 26, which is a single chain Fv fragment.

5

32. An isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;

b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; or

c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

33. The isolated human antibody, or an antigen-binding portion thereof, of claim 32 which further has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 335-SEQ ID NO: 403; or a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533.

34. The isolated human antibody, or an antigen-binding portion thereof, of claim 32 which further has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; or a light chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505.

25

35. An isolated human antibody, or an antigen-binding portion thereof, having a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

30

- 176 -

36. The isolated human antibody of claim 35, comprising a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions.

5 37. The isolated human antibody of claim 36, wherein the antibody heavy chain constant region is IgG1.

38. The isolated human antibody of claim 35, which is a Fab fragment.

10 39. The isolated human antibody of claim 35, which is a F(ab')₂ fragment.

40. The isolated human antibody of claim 35, which is a single chain Fv fragment.

15 41. An isolated human antibody, or an antigen-binding portion thereof, which
a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;

b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and

20 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

42. The isolated human antibody, or an antigen-binding portion thereof, of claim 41 which further has a heavy chain CDR2 comprising the amino acid sequence of
25 SEQ ID NO: 27; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28.

43. The isolated human antibody, or an antigen-binding portion thereof, of claim 41 which further has a heavy chain CDR1 comprising the amino acid sequence of
30 SEQ ID NO: 29; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

- 177 -

44. An isolated human antibody, or an antigen-binding portion thereof, having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

5

45. The isolated human antibody of claim 44, comprising a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions.

10

46. The isolated human antibody of claim 45, wherein the antibody heavy chain constant region is IgG1.

47. The isolated human antibody of claim 44, which is a Fab fragment.

15

48. The isolated human antibody of claim 44, which is a F(ab')₂ fragment.

49. The isolated human antibody of claim 44, which is a single chain Fv fragment.

20

50. An isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁶M or less;

25

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and

30

- 178 -

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

51. An isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}M$ or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

- 179 -

52. An isolated human antibody, or an antigen-binding portion thereof, which
- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

53. An isolated nucleic acid encoding the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17.
54. The isolated nucleic acid of claim 53, which encodes an antibody heavy chain variable region.

55. The isolated nucleic acid of claim 54, wherein the CDR2 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 19.

- 180 -

56. The isolated nucleic acid of claim 54, wherein the CDR1 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 21.

57. The isolated nucleic acid of claim 56, which encodes an antibody heavy
5 chain variable region comprising the amino acid sequence of SEQ ID NO: 23.

58. An isolated nucleic acid encoding the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18.

10 59. The isolated nucleic acid of claim 58, which encodes an antibody light chain variable region.

60. The isolated nucleic acid of claim 59, wherein the CDR2 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 20.
15

61. The isolated nucleic acid of claim 59, wherein the CDR1 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 22.

62. The isolated nucleic acid of claim 61, which encodes an antibody light
20 chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

63. An isolated human antibody, or an antigen-binding portion thereof, which
a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;

25 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold
30 higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence

- 181 -

of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and

5 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of
10 SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

64. An isolated nucleic acid encoding the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25.
15

65. The isolated nucleic acid of claim 64, which encodes an antibody heavy chain variable region.

66. The isolated nucleic acid of claim 65, wherein the CDR2 of the antibody
20 heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27.

67. The isolated nucleic acid of claim 65, wherein the CDR1 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 29.

25 68. The isolated nucleic acid of claim 67, which encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31.

69. An isolated nucleic acid encoding the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.
30

70. The isolated nucleic acid of claim 69, which encodes an antibody light chain variable region.

- 182 -

71. The isolated nucleic acid of claim 70, wherein the CDR2 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 28.

5 72. The isolated nucleic acid of claim 70, wherein the CDR1 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 30.

73. The isolated nucleic acid of claim 72, which encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

10

74. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
15 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the VH3 germline family, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing
20 amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from a member of the VL1 germline family, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing
25 amino acid residue.

- 183 -

75. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
5 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with
10 an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

15

76. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
20 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

25 c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

- 184 -

77. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which
5 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the VH3 germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other VH3 germline
10 family members, and a CDR1 that is structurally similar to CDR1s from other VH3 germline family members, and wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue;

c) has a light chain variable region comprising an amino acid sequence selected from a member of the V λ 1 germline family, wherein the light chain variable
15 region comprises a CDR2 that is structurally similar to CDR2s from other V λ 1 germline family members, and a CDR1 that is structurally similar to CDR1s from other V λ 1 germline family members, and wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

20 78 The isolated human antibody, or antigen binding portion thereof, of claims 74, 75, 76, or 77, wherein the mutation is in the heavy chain CDR3.

79. The isolated human antibody, or antigen binding portion thereof, of claims 74, 75, 76, or 77, wherein the mutation is in the light chain CDR3.

25

80. The isolated human antibody, or antigen binding portion thereof, of claims 74, 75, 76, or 77, wherein the mutation is in the heavy chain CDR2.

81. The isolated human antibody, or antigen binding portion thereof, of
30 claims 74, 75, 76, or 77, wherein the mutation is in the light chain CDR2.

- 185 -

82. The isolated human antibody, or antigen binding portion thereof, of claims 74, 75, 76, or 77, wherein the mutation is in the heavy chain CDR1.

83. The isolated human antibody, or antigen binding portion thereof, of
5 claims 74, 75, 76, or 77, wherein the mutation is in the light chain CDR1.

84. A recombinant expression vector encoding:
a) an antibody heavy chain having a variable region comprising
the amino acid sequence of SEQ ID NO: 31; and
10 b) an antibody light chain having a variable region comprising the
amino acid sequence of SEQ ID NO: 32.

85. A host cell into which the recombinant expression vector of claim 84 has
been introduced.
15

86. A method of synthesizing a human antibody that binds human IL-12,
comprising culturing the host cell of claim 85 in a culture medium until a human
antibody that binds human IL-12 is synthesized by the cell.

20 87. An isolated human antibody, or antigen-binding portion thereof, that
neutralizes the activity of human IL-12, and at least one additional primate IL-12
selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-
12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the
mouse IL-12.
25

88. A pharmaceutical composition comprising the antibody or an antigen
binding portion thereof, of any one of claims 1-52, 74-83 and 87 and a pharmaceutically
acceptable carrier.

30 89. A composition comprising the antibody or an antigen binding position
thereof, of any one of claims 1-52, 74-83 and 87 and an additional agent.

- 186 -

90. The composition of claim 89, wherein the additional agent is a therapeutic agent.

91. The composition of claim 90, wherein the therapeutic agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1 β monoclonal antibodies, anti-IL-6 monoclonal antibodies, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TNF α converting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGF β .

92. The therapeutic composition of claim 90, wherein the therapeutic agent is selected from the group consisting of anti-TNF antibodies and antibody fragments thereof, TNFR-Ig constructs, TACE inhibitors, PDE4 inhibitors, corticosteroids, budenoside, dexamethasone, sulfasalazine, 5-aminosalicylic acid, olsalazine, IL-1 β converting enzyme inhibitors, IL-1ra, tyrosine kinase inhibitors, 6-mercaptopurines and IL-11.

93. The therapeutic composition of claim 90, wherein the therapeutic agent is selected from the group consisting of corticosteroids, prednisolone, methylprednisolone, azathioprine, cyclophosphamide, cyclosporine, methotrexate, 4-aminopyridine,

- 187 -

tizanidine, interferon- β 1a, interferon- β 1b, Copolymer 1, hyperbaric oxygen, intravenous immunoglobulin, clabribine, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, PDGF, antibodies to CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their
 5 ligands, methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38 or MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TACE inhibitors, T-cell signalling inhibitors, kinase inhibitors,
 10 metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, sIL-13R, anti-P7s, p-selectin glycoprotein ligand (PSGL), antiinflammatory cytokines, IL-4, IL-10, IL-13 and TGF β .

15 94. A method for inhibiting human IL-12 activity comprising contacting human IL-12 with the antibody of claim 44 such that human IL-12 activity is inhibited.

95. A method for inhibiting human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising
 20 administering to the human subject the antibody of claim 44 such that human IL-12 activity in the human subject is inhibited.

96. The method of claim 95, wherein the disorder is selected from the group consisting of rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis,
 25 Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, ankylosing spondylitis, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, multiple sclerosis, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, thyroiditis, graft versus host disease, organ transplant rejection, acute or chronic immune disease
 30 associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, polyarteritis nodosa, Wegener's granulomatosis, Henoch-

- 188 -

Schonlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, Sjogren's syndrome, uveitis, sepsis, septic shock, sepsis syndrome, adult respiratory distress syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, myasthenia gravis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, fibrotic lung diseases, hemolytic anemia, malignancies, heart failure and myocardial infarction.

97. The method of claim 95, wherein the disorder is Crohn's disease.

98. The method of claim 95, wherein the disorder is multiple sclerosis.

99. The method of claim 95, wherein the disorder is rheumatoid arthritis.

100. A method for improving the activity of an antibody, or antigen-binding portion thereof, to attain a predetermined target activity, comprising:

a) providing a parent antibody a antigen-binding portion thereof;

b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;

d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determined if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;

e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.

- 189 -

f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.

g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result an antibody with only a partial activity, the method further comprising mutating additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determined if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, the method further comprising mutating additional amino acid residues selected from the group consisting of H33B, H52B and L31A to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

- 190 -

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

101. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
- c) individually mutating said preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
- e) repeating steps b) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position if the desired antibody activity is not obtained;
- f) combining in a stepwise fashion, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and
- g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- 191 -

102. The method of claim 101, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96.

5

103. The method of claim 101, wherein hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93.

10

104. The method of claim 101, wherein the preferred positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

15

105. The method of claim 101, wherein the contact positions are selected from the group consisting of L50 and L94.

106. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity is not further improved by mutagenesis in said phage-display system;

25 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

30

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

- 192 -

e) repeating steps b) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position if the desired antibody activity is not obtained;

f) combining, in the parent antibody, or antigen-binding portion thereof,
5 individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative
10 to the parent antibody, or antigen-binding portion thereof, is obtained.

107. The method of claim 106, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91,
15 L92, L93, L94 and L96.

108. The method of claim 106, wherein hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93.

20

109. The method of claim 106, wherein preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94

25 110. The method of claim 106, wherein the contact positions are selected from the group consisting of L50 and L94.

- 193 -

111. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof ;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation
5 position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby
10 create a panel of mutated antibodies, or antigen-binding portions thereof and expressing said panel in an appropriate expression system;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
 - 15 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained in the antibody;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at
20 least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

112. The method of claim 111, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54,
25 H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

- 194 -

113. The method of claim 111, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation
5 of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

114. The method of claim 111, wherein the preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52,
10 H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

15

115. The method of claim 111, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of
20 epitope recognition and an antibody with a close to germline immunoglobulin sequence.

116. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof;
25 that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation
30 position;

- 195 -

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
10 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective
15 mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

20 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

25

117. The method of claim 116, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from
30 the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

118. The method of claim 116, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

119. The method of claim 116 wherein the preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

120. The method of claim 116, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

121. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting a contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or

- 197 -

antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof
5 thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one additional property or characteristic, wherein the property or characteristic is one that needs to be retained,
10 until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

122. The method of claim 121, wherein contact positions are selected from the
15 group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope
20 recognition and an antibody with a close to germline immunoglobulin sequence.

123. The method of claim 121, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the
25 group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

- 198 -

124. The method of claim 121, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

125. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; 10 that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position contact or hypermutation 15 position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding 20 portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one 25 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.
- f) repeating steps a) through e) for at least one other preferred selective 30 mutagenesis position, contact or hypermutation position;
- g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity

- 199 -

and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

- h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
- 5 until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

126. The method of claim 125, wherein contact positions are selected from the
10 group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope
15 recognition and an antibody with a close to germline immunoglobulin sequence.

127. The method of claim 125, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the
20 group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

128. The method of claim 125 wherein the preferred selective mutagenesis
25 positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline
30 immunoglobulin sequence.

- 200 -

129. The method of claim 125, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

130. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- 10 b) selecting a amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- 15 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- 20 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

25

131. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- 30 b) selecting a amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

- 201 -

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, 10 L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

15 g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

20 132. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

25 b) selecting a selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

30 c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

- 202 -

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic, until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

10 133. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

15 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

25 e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

- 203 -

g) evaluating the activity and other property or characteristics of the combination antibodies, or antigen-binding portions thereof, with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

134. A method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other properties, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- 10 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- 15 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- 20 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic, wherein the property or characteristic needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and retained property, or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.
- 25

135. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- 30 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50,

- 204 -

H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity but not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof with at least one retained property or characteristic; and

h) evaluating the activity and the retention of at least one property of characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

136. A method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

- 205 -

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof
10 thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding
15 portion thereof, is obtained.

137. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was
20 obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53,
25 L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity and retention of at least one other property or
30 characteristic of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

- 206 -

e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and retention of at least one other property or
10 characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

15

138. The method of claims 130, 131, 132, 133, 134, 135, 136 or 137, wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-cross reactivity with other human tissues, preservation of epitope recognition and an antibody with a close to
20 germline immunoglobulin sequence.

139. A method for detecting human IL-12 comprising contacting human IL-12 with the antibody, or antigen-binding portion thereof, of any of claims 1-52, 74-83 and 87 such that human IL-12 is detected.

25

140. The method of claim 139, wherein human IL-12 is detected *in vitro*.

141. The method of claim 139, wherein human IL-12 is detected in a biological sample for diagnostic purposes.

30

142. Use of the antibody, or antigen-binding portion thereof, of any of claims 1-52, 74-83 and 87 in therapy.

143. Use of the antibody, or antigen-binding portion thereof, of any of claims 1-52, 74-83 and 87 in the manufacture of a medicament for the treatment of a disorder in which IL-12 activity is detrimental.

5

144. The use of claim 143, wherein the disorder is selected from the group consisting of rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, ankylosing spondylitis, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, multiple sclerosis, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis, scleroderma, thyroiditis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, polyarteritis nodosa, Wegener's granulomatosis, Henoch-Schonlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, Sjogren's syndrome, uveitis, sepsis, septic shock, sepsis syndrome, adult respiratory distress syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, myasthenia gravis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, fibrotic lung diseases, hemolytic anemia, malignancies, heart failure and myocardial infarction.

25

145. The use of claim 143, wherein the disorder is Crohn's disease.

146. The use of claim 143, wherein the disorder is multiple sclerosis.

147. The use of claim 143, wherein the disorder is rheumatoid arthritis.

1/14

Figure 1A. Heavy Chain Variable Region Sequences

SEQ ID NO:	Kabat number	CDR H1	CDR H2
33	JOE9wt VH	30 QVQLVQSGG	56 F
35	Cos-3/Jh3 VH	31 QVQLVQSGG	55 F
37	70-1 VH	32 QVQLVQSGG	54 F
39	78-34 VH	33 QVQLVQSGG	53 F
41	79-1 VH	34 QVQLVQSGG	52 F
43	101-11 VH	35 QVQLVQSGG	51 F
45	26-1 VH	36 QVQLVQSGG	50 F
47	136-15 VH	37 QVQLVQSGG	49 F
49	136-15 VH germline	38 QVQLVQSGG	48 F
51	149-5 VH	39 QVQLVQSGG	47 F
53	149-6 VH	40 QVQLVQSGG	46 F
55	103-4 VH	41 QVQLVQSGG	45 F
57	103-8 VH	42 QVQLVQSGG	44 F
59	103-14 VH	43 QVQLVQSGG	43 F
61	G6 VH	44 QVQLVQSGG	42 F
63	Y139 VH	45 QVQLVQSGG	41 F
65	AO3 VH	46 QVQLVQSGG	40 F
67	AO3 VH germline	47 QVQLVQSGG	39 F
23	Y61 VH	48 QVQLVQSGG	38 F
69	Y61 VH germline	49 QVQLVQSGG	37 F
71	Y61-H31E VH	50 QVQLVQSGG	36 F
73	Y61 L50Y VH	51 QVQLVQSGG	35 F
75	Y61-L94Y VH	52 QVQLVQSGG	34 F
31	J695	53 QVQLVQSGG	33 F

2/14

Figure 1B. Heavy Chain Variable Region Sequences

SEQ ID NO:	Kabat number	CDR H2	CDR H3	
33	JOE9wt VH	KYYADSVKG	RTISRDN SKNTLYLQMKSLRAEDTAVYYCTT	113 112 111 110 109 108 107 106 105 104 103
35	Cos-3/Jh3 VH	KYYADSVKG	RTISRDN SKNTLYLQMKSLRAEDTAVYYCTT	113 112 111 110 109 108 107 106 105 104 103
37	70-1 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
39	78-34 VH			113 112 111 110 109 108 107 106 105 104 103
41	79-1 VH			113 112 111 110 109 108 107 106 105 104 103
43	101-11 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
45	26-1 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
47	136-15 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
49	136-15 VH germline		H..H.N	113 112 111 110 109 108 107 106 105 104 103
51	149-5 VH		H..H.T	113 112 111 110 109 108 107 106 105 104 103
53	149-6 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
55	103-4 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
57	103-8 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
59	103-14 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
61	G6 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
63	Y139 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
65	AO3 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
67	AO3 VH germline		H..H.N	113 112 111 110 109 108 107 106 105 104 103
23	Y61 VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
69	Y61 VH germline		H..H.N	113 112 111 110 109 108 107 106 105 104 103
71	Y61-H31E VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
73	Y61 L50Y VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
75	Y61-L94Y VH		H..H.N	113 112 111 110 109 108 107 106 105 104 103
31	J695		H..H.N	113 112 111 110 109 108 107 106 105 104 103

4/14

Figure 1D. Light Chain Variable Region Sequences

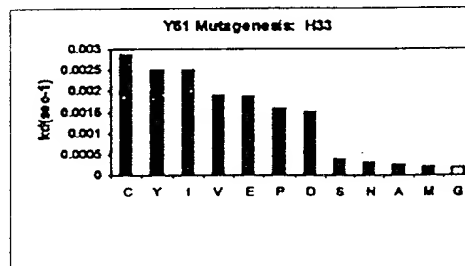
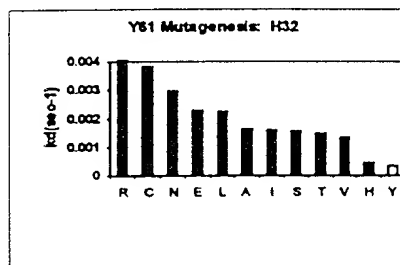
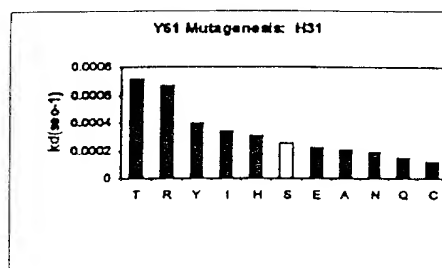
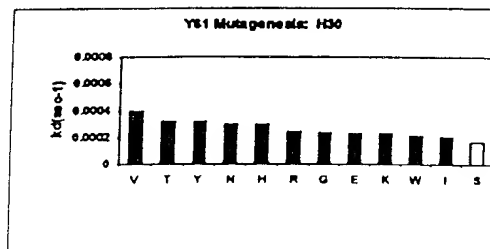
SEQ ID		Kabat number		CDR L3	
NO:		Joe9 VL wt			
34		Dp18 Lv1042/JA1			
36		70-1 VL			
38		78-34 VL			
40		79-1 VL			
42		101-11 VL			
44		26-1 VL			
46		136-15 VL			
48		136-15 VL germline			
50		149-5 VL			
52		149-6 VL			
54		103-4 VL			
56		103-8 VL			
58		103-14 VL			
60		G6 VL			
62		Y139 VL			
64		A03 VL			
66		A03 VL germline			
68		Y61 VL			
24		Y61 VL germline			
70		Y61-H31E VL			
72		Y61-L50Y VL			
74		Y61-L94Y VL			
76		J695 VL			
32					

57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067

5/14

Figure 2A. Y61 Heavy Chain CDR H1 Mutagenesis

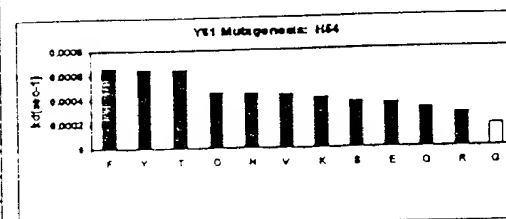
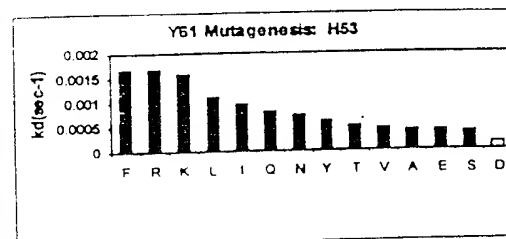
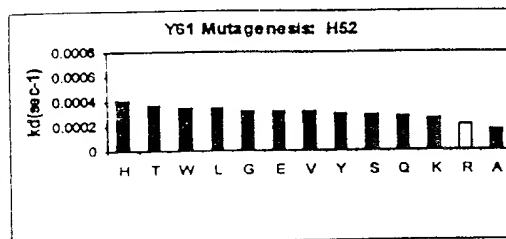
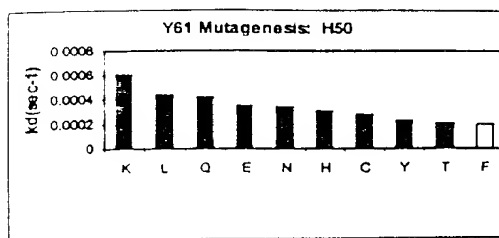
		CDR H1										
SEQ ID NO:		27	28	29	30	31	32	33	34	35	k_{off} ($\times 10^5$)	
21	Y61	F	T	F	S	S	Y	G	M	H		
288		.	.	.	E	22.8	
289		.	.	.	S	16.8	
290		.	.	.	Y	31.9	
291		.	.	.	H	29.6	
292		.	.	.	K	22.5	
293		.	.	.	R	24.5	
294		.	.	.	N	30.1	
295		.	.	.	T	32.0	
296		.	.	.	G	23.3	
297		.	.	.	V	39.9	
298		.	.	.	I	20.7	
299		.	.	.	W	21.6	
300		.	.	.	E	21.9	
301		.	.	.	C	12.0	
302		.	.	.	S	24.9	
303		.	.	.	Y	39.8	
304		.	.	.	H	30.9	
305		.	.	.	R	66.4	
306		.	.	.	N	19.1	
307		.	.	.	Q	15.2	
308		.	.	.	T	71.6	
309		.	.	.	A	20.5	
310		.	.	.	I	33.4	
311		.	.	.	E	229.0	
312		.	.	.	C	383.0	
313		.	.	.	S	157.5	
314		.	.	.	Y	33.7	
315		.	.	.	H	46.1	
316		.	.	.	R	448.5	
317		.	.	.	N	297.0	
318		.	.	.	T	148.0	
319		.	.	.	A	165.5	
320		.	.	.	V	133.5	
321		.	.	.	L	226.0	
322		.	.	.	I	160.5	
323		D	.	.	.	152.0	
324		E	.	.	.	189.0	
325		C	.	.	.	286.5	
326		S	.	.	.	39.9	
327		Y	.	.	.	250.5	
328		N	.	.	.	30.8	
329		G	.	.	.	17.8	
330		A	.	.	.	27.3	
331		V	.	.	.	191.0	
332		M	.	.	.	21.5	
333		I	.	.	.	250.0	
334		P	.	.	.	159.5	



6/14

Figure 2B. Y61 Heavy Chain CDR H2 Mutagenesis

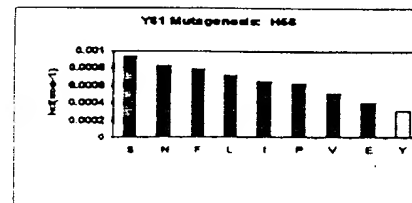
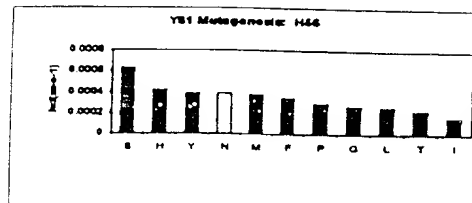
SEQ ID NO:		CDR H2																k_{off} ($\times 10^3$)	
		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64		65
19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G	
335		E	34.7
336		C	28.5
337		Y	23.0
338		H	30.9
339		K	61.2
340		N	34.4
341		Q	42.0
342		T	20.5
343		L	44.0
344		F	20.4
345		.	E	31.8
346		.	S	29.2
347		.	Y	29.8
348		.	H	40.7
349		.	K	26.2
350		.	R	20.6
351		.	Q	28.5
352		.	T	37.4
353		.	G	32.1
354		.	A	17.1
355		.	V	31.7
356		.	L	34.7
357		.	W	35.1
358		.	.	D	15.1
359		.	.	E	39.9
360		.	.	S	36.8
361		.	.	Y	61.1
362		.	.	K	158.0
363		.	.	R	166.5
364		.	.	N	72.7
365		.	.	Q	79.2
366		.	.	T	50.0
367		.	.	A	40.4
368		.	.	V	44.0
369		.	.	L	109.5
370		.	.	I	94.4
371		.	.	F	168.5
372		.	.	D	45.5
373		.	.	E	35.1
374		.	.	S	37.3
375		.	.	Y	64.6
376		.	.	K	40.7
377		.	.	R	2.5
378		.	.	N	44.7
379		.	.	Q	31.6
380		.	.	T	64.4
381		.	.	G	17.8
382		.	.	V	43.5



7/14

Figure 2C. Y61 Heavy Chain CDR H2 Mutagenesis

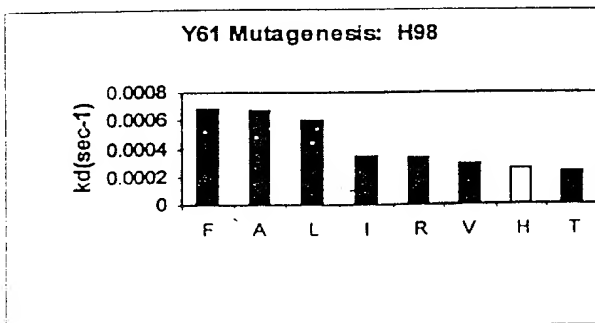
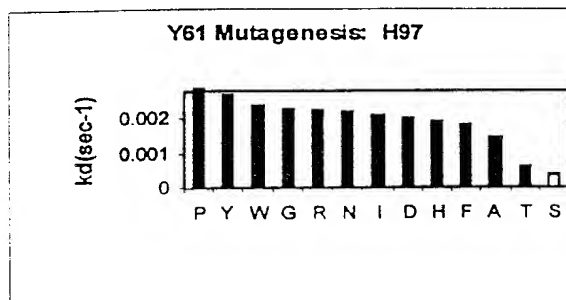
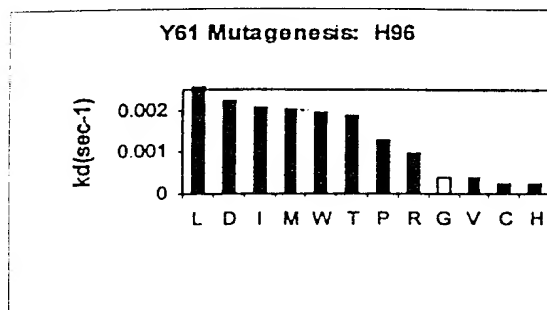
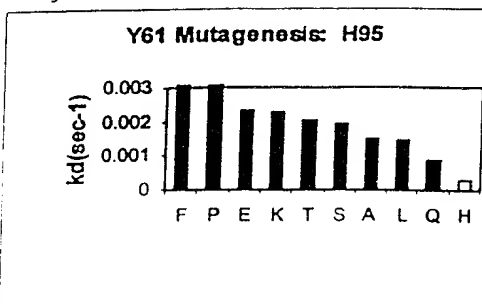
		CDR H2																	
SEQ ID NO:		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65	k_{rel} ($\times 10^3$)
19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G	
383		F	.	S	66.3
384		S	62.4
385		Y	39.0
386		H	42.0
387		N	38.5
388		T	23.5
389		G	27.2
390		M	38.3
391		L	26.4
392		I	16.9
393		P	29.9
394		F	34.5
395		E	41.5
396		S	94.1
397		Y	31.0
398		N	83.1
399		V	52.4
400		L	73.0
401		I	65.7
402		P	62.8
403		F	79.4



8/14

Figure 2D. Y61 Heavy Chain CDR H3 Mutagenesis

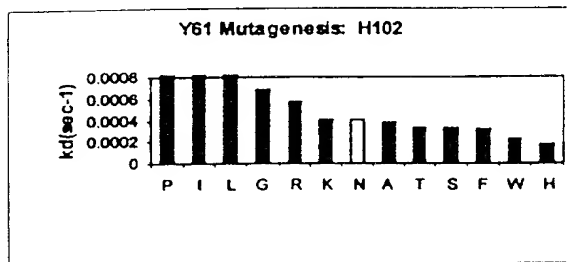
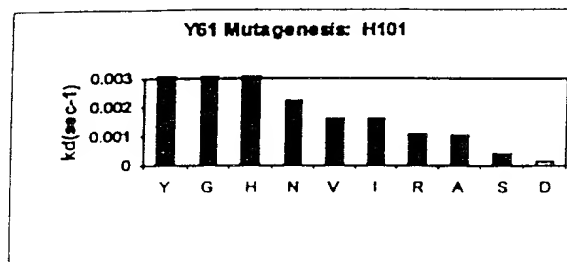
SEQ ID NO:	CDR H3							K_{off} ($\times 10^5$)
		56	96	L6	86	101	201	
17	Y61	H	G	S	H	D	N	
404		E	231.5
405		S	193.0
406		H	28.7
407		K	227.5
408		Q	85.9
409		T	202.0
410		A	150.0
411		L	147.5
412		P	471.0
413		F	514.0
414		.	D	223.5
415		.	C	24.2
416		.	H	23.7
417		.	R	96.2
418		.	T	186.0
419		.	G	39.7
420		.	V	38.2
421		.	M	204.5
422		.	L	261.0
423		.	I	207.5
424		.	P	129.0
425		.	W	197.0
426		.	.	D	.	.	.	202.0
427		.	.	S	.	.	.	37.5
428		.	.	Y	.	.	.	273.0
429		.	.	H	.	.	.	190.5
430		.	.	R	.	.	.	224.0
431		.	.	N	.	.	.	221.5
432		.	.	T	.	.	.	58.8
433		.	.	G	.	.	.	229.0
434		.	.	A	.	.	.	143.0
435		.	.	I	.	.	.	208.0
436		.	.	P	.	.	.	300.0
437		.	.	W	.	.	.	239.0
438		.	.	F	.	.	.	180.5
439		.	.	.	H	.	.	25.5
440		.	.	.	R	.	.	34.0
441		.	.	.	T	.	.	22.7
442		.	.	.	A	.	.	67.3
443		.	.	.	V	.	.	29.3
444		.	.	.	L	.	.	59.8
445		.	.	.	I	.	.	34.3
446		.	.	.	F	.	.	68.8
447		D	.	14.4
448		S	.	44.9
449		Y	.	465.0
450		H	.	327.0
451		R	.	110.0



9/14

Figure 2E. Y61 Heavy Chain CDR H3 Mutagenesis

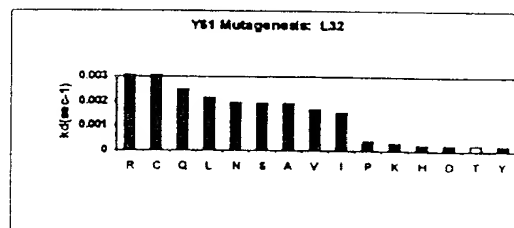
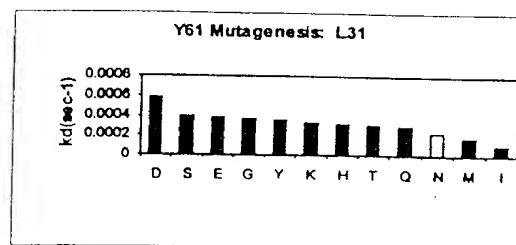
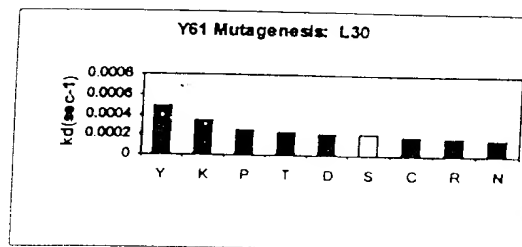
SEQ ID NO:		CDR H3						K_{off} ($\times 10^5$)
		95	96	97	98	101	102	
17	Y61	H	G	S	H	D	N	
452		N	.	223.0
453		G	.	375.0
454		A	.	106.5
455		V	.	163.0
456		I	.	162.5
457		S	32.5
458		H	18.0
459		K	40.5
460		R	57.5
461		N	40.3
462		T	33.3
463		G	69.2
464		A	38.2
465		L	95.6
466		I	99.6
467		P	181.5
468		W	23.5
469		F	31.8



10/14

Figure 2F. Y61 Light Chain CDR L1 Mutagenesis

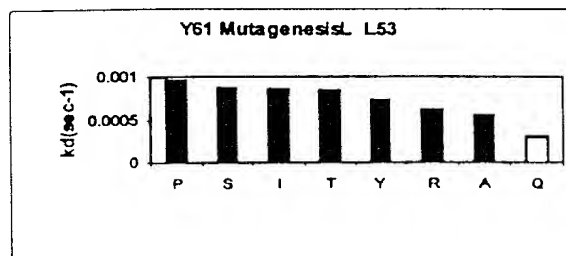
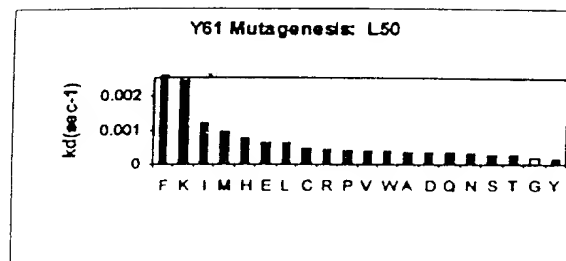
SEQ ID NO:	CDR L1														k_{off} ($\times 10^5$)
	Y61	24	25	26	27	27A	27B	28	29	30	31	32	33	34	
22	Y61	S	G	G	R	S	N	I	G	S	N	T	V	K	
470		D	22.0
471		C	18.6
472		S	21.1
473		Y	48.3
474		K	34.6
475		R	18.2
476		N	16.6
477		T	22.6
478		P	25.0
479		D	58.0
480		E	38.4
481		S	39.2
482		Y	35.7
483		H	31.5
484		K	33.1
485		N	22.9
486		Q	29.2
487		T	30.9
488		G	36.6
489		M	17.4
490		I	9.7
491		D	25.2
492		C	381.5
493		S	191.0
494		Y	21.3
495		H	26.0
496		K	31.8
497		R	690.0
498		N	196.5
499		Q	247.0
500		T	24.1
501		A	190.5
502		V	164.5
503		L	215.5
504		I	154.0
505		P	42.4



11/14

Figure 2G. Y61 Light Chain CDR L2 Mutagenesis

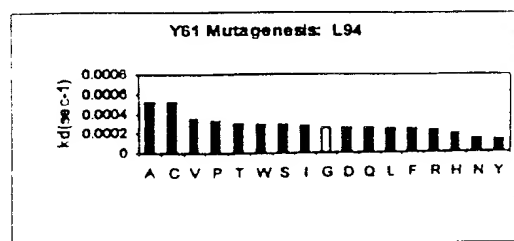
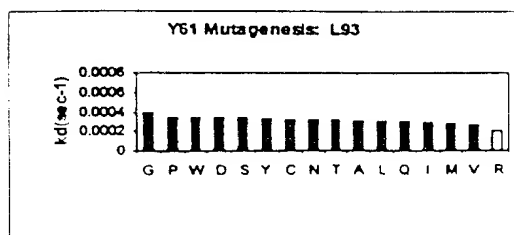
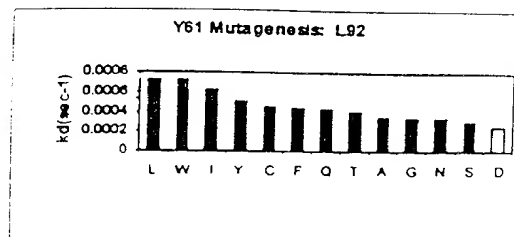
SEQ ID NO:		CDR L2							K_{off} ($\times 10^5$)
		50	51	52	53	54	55	56	
20	Y61	G	N	D	Q	R	P	S	
506		D	34.8
507		E	61.7
508		C	46.7
509		S	28.6
510		Y	17.4
511		H	76.1
512		K	242.5
513		R	44.4
514		N	30.5
515		Q	34.8
516		T	27.2
517		G	21.5
518		A	37.2
519		V	38.5
520		M	95.3
521		L	61.6
522		I	120.5
523		P	41.0
524		W	38.2
525		F	3,476.7
526		.	.	.	S	.	.	.	86.6
527		.	.	.	Y	.	.	.	73.3
528		.	.	.	R	.	.	.	61.4
529		.	.	.	Q	.	.	.	29.7
530		.	.	.	T	.	.	.	83.4
531		.	.	.	A	.	.	.	55.4
532		.	.	.	I	.	.	.	85.5
533		.	.	.	P	.	.	.	97.4



12/14

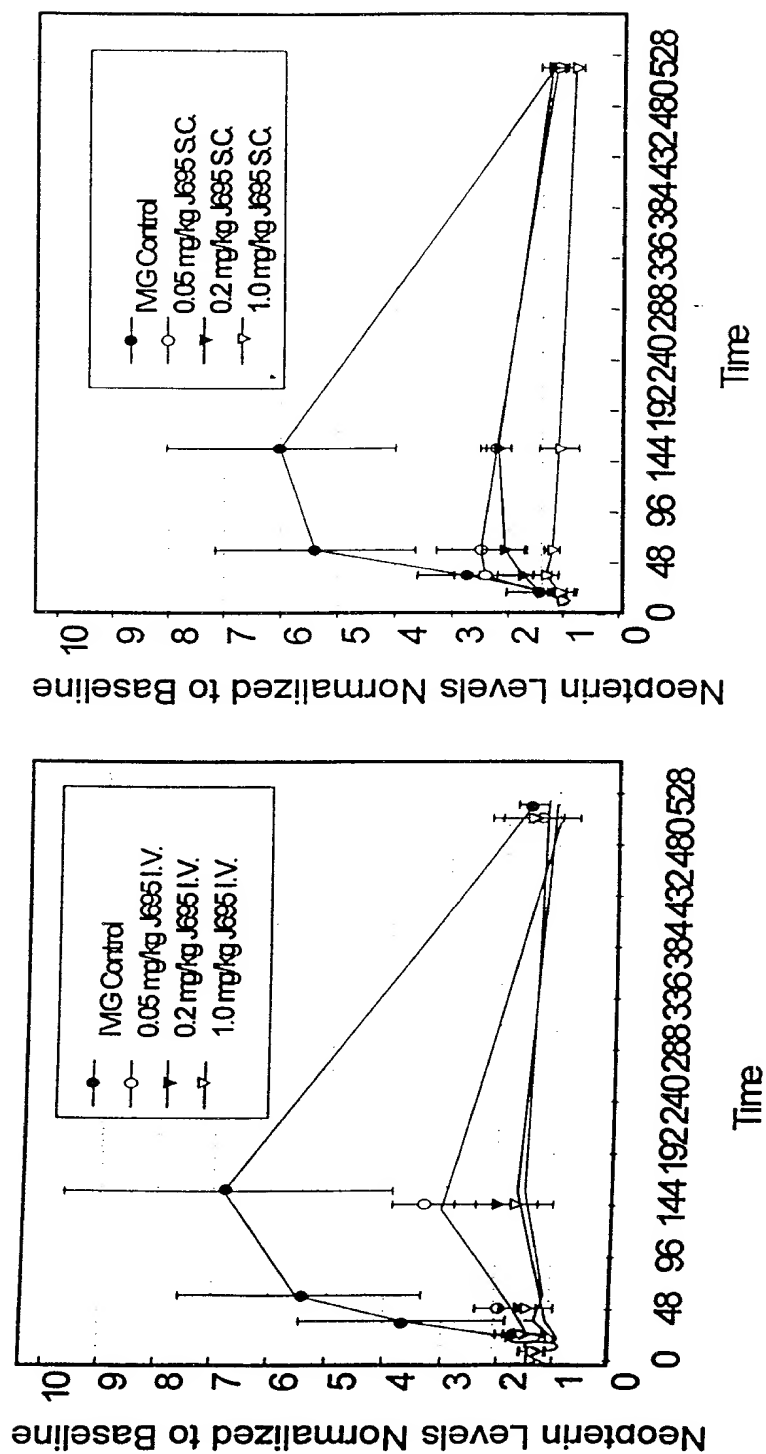
Figure 2H. Y61 Light Chain CDR L3 Mutagenesis

SEQ ID NO:	CDR L3													K_{off} ($\times 10^5$)
		89	90	91	92	93	94	95	95A	95B	95C	96	97	
18	Y61	Q	S	Y	D	R	G	T	H	P	A	L	L	
534		.	.	.	D	25.9
535		.	.	.	C	45.3
536		.	.	.	S	30.7
537		.	.	.	Y	51.1
538		.	.	.	N	34.7
539		.	.	.	Q	42.7
540		.	.	.	T	40.8
541		.	.	.	G	34.9
542		.	.	.	A	35.7
543		.	.	.	L	72.8
544		.	.	.	I	61.8
545		.	.	.	W	72.0
546		.	.	.	F	44.9
547		.	.	.	D	34.3
548		.	.	.	C	32.0
549		.	.	.	S	34.1
550		.	.	.	Y	33.5
551		.	.	.	R	19.9
552		.	.	.	N	31.6
553		.	.	.	Q	30.0
554		.	.	.	T	31.6
555		.	.	.	G	39.2
556		.	.	.	A	31.0
557		.	.	.	V	26.9
558		.	.	.	M	27.5
559		.	.	.	L	30.0
560		.	.	.	I	29.5
561		.	.	.	P	34.9
562		.	.	.	W	34.9
563		.	.	.	D	25.3
564		.	.	.	C	52.0
565		.	.	.	S	28.7
566		.	.	.	Y	13.1
567		.	.	.	H	18.7
568		.	.	.	R	23.1
569		.	.	.	N	13.7
570		.	.	.	Q	25.0
571		.	.	.	T	30.5
572		.	.	.	G	25.6
573		.	.	.	A	52.6
574		.	.	.	V	35.1
575		.	.	.	L	24.4
576		.	.	.	I	27.6
577		.	.	.	P	33.2
578		.	.	.	W	29.3
579		.	.	.	F	23.6



13/14

Figure 3: *In vivo* efficacy of J695 in cynomolgus monkeys



14/14

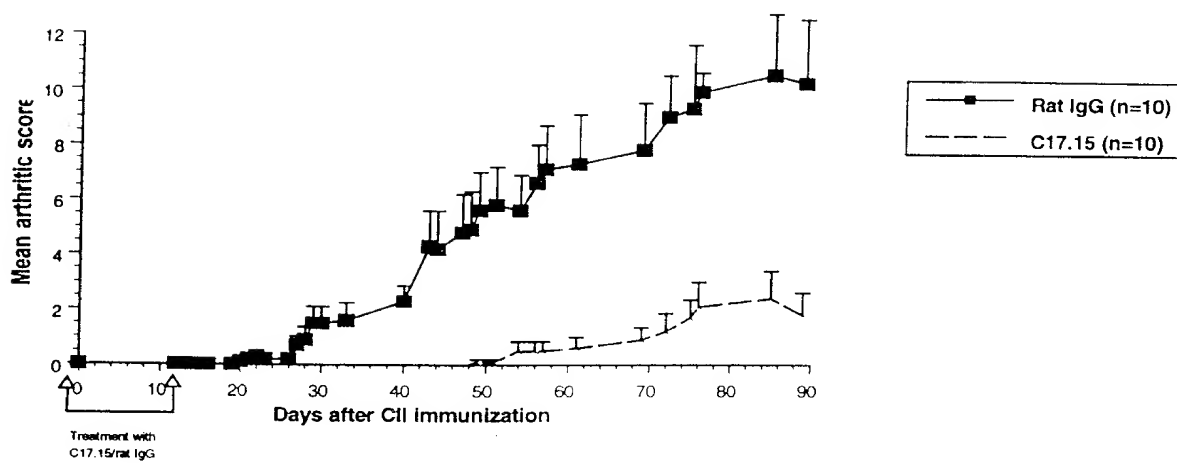


FIGURE 4

- 1 -

SEQUENCE LISTING

<110> Jochen, Salfeld et al.

<120> Human Antibodies That Bind Human IL-12 And Methods For Producing

<130> BBI-093CPPC

<140>

<141>

<150> 60/126,603

<151> March 25, 1999

<160> 675

<170> PatentIn Ver. 2.0

<210> 1

<211> 6

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 could be either His or Ser

<220>

<223> Xaa at position 4 could be either Tyr or His

<220>

<223> Xaa at position 6 could be either Tyr, Asn or Thr

<400> 1

Xaa Gly Ser Xaa Asp Xaa

1 5

<210> 2

<211> 12

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 2 could be either Ser or Thr

<220>

<223> Xaa at position 4 could be either Asp or Glu

<220>

<223> Xaa at position 5 could be either Ser, Arg or Lys

<220>

<223> Xaa at position 6 could be either Ser, Gly or Tyr

<220>

<223> Xaa at position 7 could be either Leu, Phe, Thr or Ser

<220>

<223> Xaa at position 8 could be either Arg, Ser, Thr, Trp or His

<220>

<223> Xaa at position 9 could be either Gly or Pro

<220>

- 2 -

<223> Xaa at position 10 could be either Ser, Thr, Ala
or Leu

<220>

<223> Xaa at position 11 could be either Arg, Ser, Met,
Thr or Leu

<220>

<223> Xaa at position 12 could be either Val, Ile, Thr,
Met or Leu

<400> 2

Gln Xaa Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10

<210> 3

<211> 17

<212> PRT

<213> Homo sapiens

<400> 3

Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 4

<211> 7

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 could be either Gly or Tyr

<220>

<223> Xaa at position 3 could be either Asp or Ser

<220>

<223> Xaa at position 4 could be either Gln or Asn

<400> 4

Xaa Asn Xaa Xaa Arg Pro Ser
1 5

<210> 5

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa represents either Ser or Glu

<400> 5

Phe Thr Phe Ser Xaa Tyr Gly Met His
1 5

<210> 6

<211> 13

<212> PRT

<213> Homo sapiens

- 3 -

<220>
 <223> Xaa at position 1 could be either Ser or Thr

 <220>
 <223> Xaa at position 3 could be either Ser or Gly

 <220>
 <223> Xaa at position 4 could be either Arg or Ser

 <220>
 <223> Xaa at position 8 could be either Gly or Val

 <220>
 <223> Xaa at position 9 could be either Ser or Ala

 <220>
 <223> Xaa at position 10 could be either Asn, Gly or Tyr

 <220>
 <223> Xaa at position 11 could be either Thr or Asp

 <220>
 <223> Xaa at position 13 could be either Lys or His

 <400> 6
 Xaa Gly Xaa Xaa Ser Asn Ile Xaa Xaa Xaa Xaa Val Xaa
 1 5 10

<210> 7
 <211> 115
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Xaa at position 6 could be either Gln or Glu

 <220>
 <223> Xaa at position 16 could be either Arg or Gly

 <220>
 <223> Xaa at position 31 could be either Ser or Glu

 <220>
 <223> Xaa at position 84 could be either Lys or Asn

 <220>
 <223> Xaa at position 97 could be either Thr, Ala or Lys

 <220>
 <223> Xaa at position 98 could be either Thr or Lys

 <220>
 <223> Xaa at position 99 could be either Ser or His

 <220>
 <223> Xaa at position 102 could be either Tyr or His

 <220>
 <223> Xaa at position 104 could be either Tyr, Asn or Thr

 <400> 7
 Gln Val Gln Leu Val Xaa Ser Gly Gly Gly Val Val Gln Pro Gly Xaa
 1 5 10 15

- 4 -

```

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr
      20              25              30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35              40              45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Asx
      50              55              60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65              70              75              80
Leu Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85              90              95
Xaa Xaa Xaa Gly Ser Xaa Asp Xaa Trp Gly Gln Gly Thr Met Val Thr
      100             105             110
Val Ser Ser
      115

```

<210> 8
 <211> 112
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Xaa at position 1 could be either Ser or Gln

 <220>
 <223> Xaa at position 2 could be either Tyr or Ser

 <220>
 <223> Xaa at position 13 could be either Thr or Ala

 <220>
 <223> Xaa at position 23 and 91 could be either Ser or Thr

 <220>
 <223> Xaa at position 25 could be either Gly or Ser

 <220>
 <223> Xaa at position 26 could be either Arg or Ser

 <220>
 <223> Xaa at position 30 could be either Gly or Val

 <220>
 <223> Xaa at position 31 could be either Ser or Ala

 <220>
 <223> Xaa at position 35 could be either Lys or His

 <220>
 <223> Xaa at position 51 could be either Gly or Lys

 <220>
 <223> Xaa at position 54 could be either Gln or Asn

 <220>
 <223> Xaa at position 79 could be either Val or Leu

 <220>
 <223> Xaa at position 93 could be either Asp or Glu

- 5 -

<220>
 <223> Xaa at position 94 could be either Ser, Arg or Lys

 <220>
 <223> Xaa at position 95 could be either Ser, Gly or Tyr

 <220>
 <223> Xaa at position 96 could be either Leu, Phe, Thr
 or Ser

 <220>
 <223> Xaa at position 97 could be either Arg, Ser, Thr,
 Trp or His

 <220>
 <223> Xaa at position 98 could be either Gly or Pro

 <220>
 <223> Xaa at position 99 could be either Ser, Thr, Ala
 or Leu

 <220>
 <223> Xaa at position 100 could be either Arg, Ser, Met,
 Thr or Leu

 <220>
 <223> Xaa at position 101 could be either Val, Ile, Thr,
 Met or Leu

 <220>
 <223> Xaa at position 32 could be either Asn, Gly or Tyr

 <220>
 <223> Xaa at position 33 could be either Thr or Asp

 <220>
 <223> Xaa at position 53 could be either Asp or Ser

 <400> 8
 Xaa Xaa Val Leu Thr Gln Pro Pro Ser Val Ser Gly Xaa Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Xaa Gly Xaa Xaa Ser Asn Ile Xaa Xaa Xaa
 20 25 30
 Xaa Val Xaa Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Xaa Asn Xaa Xaa Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Xaa Tyr Xaa Xaa Xaa Xaa
 85 90 95
 Xaa Xaa Xaa Xaa Xaa Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 9

- 6 -

<211> 6
<212> PRT
<213> Homo sapiens

<220>
<223> Xaa at position 2 could be either Gly, Val, Cys or His

<220>
<223> Xaa at position 3 could be either Ser or Thr

<220>
<223> Xaa at position 4 could be either His, Thr, Val, Arg, or Ile

<220>
<223> Xaa at position 5 could be either Asp or Ser

<220>
<223> Xaa at position 6 could be either Asn, Lys, Ala, Thr, Ser, Phe, Trp, or His

<400> 9
His Xaa Xaa Xaa Xaa Xaa
1 5

<210> 10
<211> 12
<212> PRT
<213> Homo sapiens

<220>
<223> Xaa at position 4 could be either Asp or Ser

<220>
<223> Xaa at position 5 represents any amino acid

<220>
<223> Xaa at position 6 could be either Gly, Asp, Gln, Leu, Phe, Arg, His, Asn or Tyr

<400> 10
Gln Ser Tyr Xaa Xaa Xaa Thr His Pro Ala Leu Leu
1 5 10

<210> 11
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<223> Xaa at position 1 could be either Phe, Thr or Tyr

<220>
<223> Xaa at position 3 could be either Arg or Ala

<220>
<223> Xaa at position 5 could be either Asp, Ser, Glu or Ala

<220>
<223> Xaa at position 6 could be either Gly or Arg

<220>
<223> Xaa at position 8 represents any amino acid

- 7 -

<220>

<223> Xaa at position 10 could be either Tyr or Glu

<400> 11

Xaa	Ile	Xaa	Tyr	Xaa	Xaa	Ser	Xaa	Lys	Xaa	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 12

<211> 7

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 could be either Gly, Tyr, Ser,
Thr, Asn or Gln

<400> 12

Xaa	Asn	Asp	Gln	Arg	Pro	Ser
1				5		

<210> 13

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 4 and 5 represents any amino acid

<220>

<223> Xaa at position 6 could be either Tyr or His

<220>

<223> Xaa at position 7 could be either Gly, Met, Ala,
Asn or Ser

<400> 13

Phe	Thr	Phe	Xaa	Xaa	Xaa	Met	His
1				5			

<210> 14

<211> 13

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 9 could be either Ser, Cys, Arg,
Asn, Asp or Thr

<220>

<223> Xaa at position 10 could be either Asn, Met or Ile

<220>

<223> Xaa at position 11 could be either Thr, Tyr, Asp,
His, Lys or Pro

<400> 14

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Xaa	Xaa	Xaa	Val	Lys
1					5					10		

- 8 -

<210> 15
 <211> 114
 <212> PRT
 <213> Homo sapiens

 <220>
 <223> Xaa at position 30 could be Ser or Glu

 <220>
 <223> Xaa at position 83 could be Lys or Asn

 <220>
 <223> Xaa at position 5 could be either Gln or Glu

 <400> 15
 Gln Val Gln Val Xaa Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
 1 5 10 15
 Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr Gly
 20 25 30
 Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
 35 40 45
 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
 65 70 75 80
 Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Lys
 85 90 95
 Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr Val
 100 105 110

 Ser Ser

<210> 16
 <211> 112
 <212> PRT
 <213> Homo sapiens

 <220>
 <223> Xaa at position 1 could be either Ser or Gln

 <220>
 <223> Xaa at position 2 could be Tyr or Ser

 <220>
 <223> Xaa at position 13 could be either Thr or Ala

 <220>
 <223> Xaa at position 25 could be either Gly or Ser

 <220>
 <223> Xaa at position 51 and 95 could be either Gly or Tyr

 <220>
 <223> Xaa at position 79 could be either Val or Leu

 <400> 16

- 9 -

Xaa Xaa Val Leu Thr Gln Pro Pro Ser Val Ser Gly Xaa Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Xaa Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Xaa Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Xaa Thr
 85 90 95
 His Pro Ala Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 17
 <211> 6
 <212> PRT
 <213> Homo sapiens

<400> 17
 His Gly Ser His Asp Asn
 1 5

<210> 18
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 18
 Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 19
 <211> 17
 <212> PRT
 <213> Homo sapiens

<400> 19
 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 20
 <211> 7
 <212> PRT
 <213> Homo sapiens

<400> 20
 Gly Asn Asp Gln Arg Pro Ser
 1 5

- 10 -

<210> 21
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 21
 Phe Thr Phe Ser Ser Tyr Gly Met His
 1 5

<210> 22
 <211> 13
 <212> PRT
 <213> Homo sapiens

<400> 22
 Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Thr Val Lys
 1 5 10

<210> 23
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 23
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 24
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 24
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val, Thr Ile Ser Cys Ser Gly Gly Arg Ser Trp Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu

- 11 -

35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
 85 90 95
 His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 25
 <211> 6
 <212> PRT
 <213> Homo sapiens

<400> 25
 His Gly Ser His Asp Asn
 1 5

<210> 26
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 26
 Gln Ser Tyr Asp Arg Tyr Thr His Pro Ala Leu Leu
 1 5 10

<210> 27
 <211> 17
 <212> PRT
 <213> Homo sapiens

<400> 27
 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 28
 <211> 7
 <212> PRT
 <213> Homo sapiens

<400> 28
 Tyr Asn Asp Gln Arg Pro Ser
 1 5

<210> 29
 <211> 9
 <212> PRT
 <213> Homo sapiens

- 12 -

<400> 29

Phe Thr Phe Ser Ser Tyr Gly Met His
1 5

<210> 30

<211> 13

<212> PRT

<213> Homo sapiens

<400> 30

Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn Thr Val Lys
1 5 10

<210> 31

<211> 115

<212> PRT

<213> Homo sapiens

<400> 31

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110Val Ser Ser
115

<210> 32

<211> 112

<212> PRT

<213> Homo sapiens

<400> 32

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
20 25 30Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45Ile Tyr Tyr Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80

- 13 -

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Tyr Thr
 85 90 95

His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 33
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 33
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Thr Thr Ser Gly Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

Val Ser Ser
 115

<210> 34
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 34
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95

- 14 -

Arg Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 35
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 35
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Ser Gly Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 36
 <211> 112
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Xaa at position 32 represents either Gly or Tyr

<400> 36
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Xaa
 20 25 30
 Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Ser Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly

- 15 -

100

105

110

<210> 37
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 37
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 38
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 38
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Arg Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

- 16 -

<210> 39
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 39
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr Ser Gly Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 40
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 40
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
 85 90 95
 Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 41
 <211> 115

- 17 -

<212> PRT

<213> Homo sapiens

<400> 41

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr Ser Gly Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 42

<211> 112

<212> PRT

<213> Homo sapiens

<400> 42

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Trp Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 43

<211> 115

<212> PRT

<213> Homo sapiens

<400> 43

- 18 -

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 44
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 44
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
 85 90 95
 Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 45
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 45
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr

- 19 -

20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 46
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 46
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Trp Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 47
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 47
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

- 20 -

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

Val Ser Ser
 115

<210> 48
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 48
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
 85 90 95

Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 49
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 49
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

- 21 -

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

Val Ser Ser
 115

<210> 50
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 50
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
 85 90 95

Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 51
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 51
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

- 22 -

85 90 95
 Thr Thr His Gly Ser His Asp Thr Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

Val Ser Ser
 115

<210> 52
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 52
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Trp Gly Thr Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 53
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 53
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

- 23 -

Val Ser Ser
115

<210> 54
<211> 112
<212> PRT
<213> Homo sapiens

<400> 54
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Val Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
85 90 95
Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 55
<211> 115
<212> PRT
<213> Homo sapiens

<400> 55
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

- 24 -

<210> 56
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 56
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Val Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
 85 90 95
 Thr Gly Ala Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 57
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 57
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 58
 <211> 112
 <212> PRT

- 25 -

<213> Homo sapiens

<400> 58

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
 85 90 95
 Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 59

<211> 115

<212> PRT

<213> Homo sapiens

<400> 59

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 60

<211> 112

<212> PRT

<213> Homo sapiens

<400> 60

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln

- 26 -

```

      1             5             10             15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
      20             25             30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35             40             45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
      50             55             60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
      65             70             75             80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Glu Arg Gly Phe
      85             90             95
Thr Gly Ser Met Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
      100            105            110

```

<210> 61
 <211> 115
 <212> PRT
 <213> Homo sapiens

```

<400> 61
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1             5             10             15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20             25             30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35             40             45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50             55             60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65             70             75             80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85             90             95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
      100            105            110
Val Ser Ser
      115

```

<210> 62
 <211> 112
 <212> PRT
 <213> Homo sapiens

```

<400> 62
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
  1             5             10             15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
      20             25             30

```

Thr	Val	Lys	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu
		35					40					45			
Ile	Tyr	Gly	Asn	Asp	Gln	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser
	50					55					60				
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Thr	Gly	Val	Gln
	65				70					75					80
Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Arg	Gly	Thr
				85					90					95	
His	Pro	Leu	Thr	Ile	Phe	Gly	Thr	Gly	Thr	Lys	Val	Thr	Val	Leu	Gly
			100					105					110		

```

<400> 63
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                    5                    10                    15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                    25                    30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                    40                    45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                    55                    60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                    70                    75                    80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                    90                    95

Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
      100                    105                    110

Val Ser Ser
      115

```

```

<400> 64
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1          5          10         15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
          20         25         30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35          40         45

```

- 28 -

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Ser
 85 90 95
 His Pro Ala Leu Thr Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 65
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 65
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 66
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 66
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln

65					70					75					80
Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Arg	Gly	Thr
				85					90					95	
His	Pro	Leu	Thr	Met	Phe	Gly	Thr	Gly	Thr	Lys	Val	Thr	Val	Leu	Gly
			100					105					110		

```
<210> 67
<211> 115
<212> PRT
<213> Homo sapiens
```

```

<400> 67
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                               5                               10                               15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                               25                               30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                               40                               45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                               55                               60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                               70                               75                               80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                               90                               95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
      100                               105                               110
Val Ser Ser
      115

```

```
<210> 68
<211> 112
<212> PRT
<213> Homo sapiens
```

```

<400> 68
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
  1                    5                10              15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
      20                25              30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35                40              45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
      50                55              60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
  65                70                75              80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
      85                90              95

```


- 30 -

His Pro Leu Thr Met Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 69
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 69
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 70
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 70
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
 85 90 95
 His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

- 31 -

<210> 71
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 71
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 72
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 72
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
 85 90 95
 His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

- 32 -

<210> 73
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 73
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 74
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 74
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Tyr Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
 85 90 95
 His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 75
 <211> 115
 <212> PRT
 <213> Homo sapiens

- 33 -

<400> 75

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110

Val Ser Ser
 115

<210> 76

<211> 112

<212> PRT

<213> Homo sapiens

<400> 76

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
 65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Tyr Thr
 85 90 95

His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 77

<211> 6

<212> PRT

<213> Homo sapiens

<400> 77

Ser Gly Ser Tyr Asp Tyr
 1 5

- 34 -

<210> 78
<211> 6
<212> PRT
<213> Homo sapiens

<400> 78
His Gly Ser His Asp Asn
1 5

<210> 79
<211> 6
<212> PRT
<213> Homo sapiens

<400> 79
His Gly Ser Tyr Asp Tyr
1 5

<210> 80
<211> 6
<212> PRT
<213> Homo sapiens

<400> 80
Arg Arg Arg Ser Asn Tyr
1 5

<210> 81
<211> 6
<212> PRT
<213> Homo sapiens

<400> 81
Ser Gly Ser Ile Asp Tyr
1 5

<210> 82
<211> 6
<212> PRT
<213> Homo sapiens

<400> 82
His Gly Ser His Asp Asp
1 5

<210> 83
<211> 6
<212> PRT
<213> Homo sapiens

<400> 83
His Gly Ser His Asp Asn
1 5

<210> 84
<211> 12
<212> PRT
<213> Homo sapiens

- 35 -

<400> 84
Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly
1 5 10

<210> 85
<211> 12
<212> PRT
<213> Homo sapiens

<400> 85
Ala Lys His Gly Ser His Asp Asn Trp Gly Gln Gly
1 5 10

<210> 86
<211> 12
<212> PRT
<213> Homo sapiens

<400> 86
Thr Thr His Gly Ser His Asp Asn Trp Ser Gln Gly
1 5 10

<210> 87
<211> 12
<212> PRT
<213> Homo sapiens

<400> 87
Thr Thr His Gly Ser His Asp Thr Trp Gly Gln Gly
1 5 10

<210> 88
<211> 12
<212> PRT
<213> Homo sapiens

<400> 88
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly
1 5 10

<210> 89
<211> 12
<212> PRT
<213> Homo sapiens

<400> 89
Lys Thr His Gly Ser His Asp Asn Trp Gly His Gly
1 5 10

<210> 90
<211> 12
<212> PRT
<213> Homo sapiens

<400> 90
Thr Thr His Gly Ser His Asp Asn Trp Ser Gln Gly
1 5 10

- 36 -

<210> 91
<211> 12
<212> PRT
<213> Homo sapiens

<400> 91
Thr Thr His Arg Ser His Asn Asn Trp Gly Gln Gly
1 5 10

<210> 92
<211> 8
<212> PRT
<213> Homo sapiens

<400> 92
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 93
<211> 8
<212> PRT
<213> Homo sapiens

<400> 93
Thr Thr His Gly Ser His Asp Thr
1 5

<210> 94
<211> 8
<212> PRT
<213> Homo sapiens

<400> 94
Thr Lys His Gly Ser His Asp Asn
1 5

<210> 95
<211> 8
<212> PRT
<213> Homo sapiens

<400> 95
Thr Thr Gln Gly Arg His Asp Asn
1 5

<210> 96
<211> 8
<212> PRT
<213> Homo sapiens

<400> 96
Lys Thr Arg Gly Arg His Asp Asn
1 5

<210> 97
<211> 8
<212> PRT
<213> Homo sapiens

- 37 -

<400> 97
Thr Thr His Gly Ser His Asp Lys
1 5

<210> 98
<211> 8
<212> PRT
<213> Homo sapiens

<400> 98
Thr Thr His Gly Ser His Asp Asp
1 5

<210> 99
<211> 8
<212> PRT
<213> Homo sapiens

<400> 99
Lys Thr His Gly Ser His Asp Asn
1 5

<210> 100
<211> 8
<212> PRT
<213> Homo sapiens

<400> 100
Lys Thr His Gly Ser His Asp Asn
1 5

<210> 101
<211> 8
<212> PRT
<213> Homo sapiens

<400> 101
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 102
<211> 8
<212> PRT
<213> Homo sapiens

<400> 102
Thr Thr Ser Gly Ser Tyr Asp Tyr
1 5

<210> 103
<211> 8
<212> PRT
<213> Homo sapiens

<400> 103
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 104

- 38 -

<211> 8
<212> PRT
<213> Homo sapiens

<400> 104
Thr Thr His Gly Ser Gln Asp Asn
1 5

<210> 105
<211> 8
<212> PRT
<213> Homo sapiens

<400> 105
Ala Thr His Gly Ser Gln Asp Asn
1 5

<210> 106
<211> 6
<212> PRT
<213> Homo sapiens

<400> 106
His Gly Ser Gln Asp Thr
1 5

<210> 107
<211> 6
<212> PRT
<213> Homo sapiens

<400> 107
Ser Gly Ser Tyr Asp Tyr
1 5

<210> 108
<211> 6
<212> PRT
<213> Homo sapiens

<400> 108
His Gly Ser Gln Asp Asn
1 5

<210> 109
<211> 9
<212> PRT
<213> Homo sapiens

<400> 109
Cys Lys Thr His Gly Ser His Asp Asn
1 5

<210> 110
<211> 12
<212> PRT
<213> Homo sapiens

<400> 110
Gln Ser Tyr Asp Ser Ser Leu Arg Gly Ser Arg Val

- 39 -

1 5 10

<210> 111
<211> 12
<212> PRT
<213> Homo sapiens

<400> 111
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
1 5 10

<210> 112
<211> 12
<212> PRT
<213> Homo sapiens

<400> 112
Gln Ser Tyr Asp Ser Ser Leu Arg Gly Ser Arg Val
1 5 10

<210> 113
<211> 12
<212> PRT
<213> Homo sapiens

<400> 113
Gln Ser Tyr Asp Ser Ser Leu Thr Gly Ser Arg Val
1 5 10

<210> 114
<211> 12
<212> PRT
<213> Homo sapiens

<400> 114
Gln Ser Tyr Asp Ser Ser Leu Trp Gly Ser Arg Val
1 5 10

<210> 115
<211> 12
<212> PRT
<213> Homo sapiens

<400> 115
Gln Thr Tyr Asp Ile Ser Glu Ser Gly Ser Arg Val
1 5 10

<210> 116
<211> 12
<212> PRT
<213> Homo sapiens

<400> 116
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
1 5 10

<210> 117
<211> 12
<212> PRT

- 40 -

<213> Homo sapiens

<400> 117

Gln	Thr	Tyr	Asp	Arg	Gly	Phe	Thr	Gly	Ser	Arg	Val
1				5					10		

<210> 118

<211> 12

<212> PRT

<213> Homo sapiens

<400> 118

Gln	Thr	Tyr	Asp	Lys	Gly	Phe	Thr	Gly	Ser	Ser	Val
1				5					10		

<210> 119

<211> 12

<212> PRT

<213> Homo sapiens

<400> 119

Gln	Ser	Tyr	Asp	Arg	Arg	Phe	Thr	Gly	Ser	Arg	Val
1				5					10		

<210> 120

<211> 12

<212> PRT

<213> Homo sapiens

<400> 120

Gln	Ser	Tyr	Asp	Trp	Asn	Phe	Thr	Gly	Ser	Arg	Val
1				5					10		

<210> 121

<211> 12

<212> PRT

<213> Homo sapiens

<400> 121

Gln	Ser	Tyr	Asp	Arg	Gly	Phe	Thr	Gly	Ser	Arg	Val
1				5					10		

<210> 122

<211> 12

<212> PRT

<213> Homo sapiens

<400> 122

Gln	Ser	Tyr	Asp	Asn	Gly	Phe	Thr	Gly	Ser	Arg	Val
1				5					10		

<210> 123

<211> 12

<212> PRT

<213> Homo sapiens

<400> 123

Gln	Ser	Tyr	Asp	Asn	Ala	Val	Thr	Ala	Ser	Lys	Val
1				5					10		

- 41 -

<210> 124
<211> 12
<212> PRT
<213> Homo sapiens

<400> 124
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
1 5 10

<210> 125
<211> 12
<212> PRT
<213> Homo sapiens

<400> 125
Gln Ser Tyr Asp Ser Ser Leu Trp Gly Thr Arg Val
1 5 10

<210> 126
<211> 12
<212> PRT
<213> Homo sapiens

<400> 126
Gln Ser Tyr Asp Arg Asp Phe Thr Gly Ser Arg Val
1 5 10

<210> 127
<211> 12
<212> PRT
<213> Homo sapiens

<400> 127
Gln Ser Tyr Glu Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 128
<211> 12
<212> PRT
<213> Homo sapiens

<400> 128
Gln Ser Tyr Asp Asn Gly Phe Thr Gly Ala Arg Val
1 5 10

<210> 129
<211> 12
<212> PRT
<213> Homo sapiens

<400> 129
Gln Ser Tyr Asp Arg Arg Phe Thr Gly Ser Arg Val
1 5 10

<210> 130
<211> 12
<212> PRT

- 42 -

<213> Homo sapiens

<400> 130

Gln	Thr	Tyr	Asp	Lys	Gly	Phe	Thr	Gly	Ser	Ser	Val
1				5					10		

<210> 131

<211> 12

<212> PRT

<213> Homo sapiens

<400> 131

Gln	Ser	Tyr	Asp	Arg	Asp	Phe	Thr	Gly	Thr	Arg	Val
1				5					10		

<210> 132

<211> 12

<212> PRT

<213> Homo sapiens

<400> 132

Gln	Ser	Tyr	Asp	Arg	Gly	Phe	Tyr	Gly	Ser	Met	Val
1				5					10		

<210> 133

<211> 12

<212> PRT

<213> Homo sapiens

<400> 133

Gln	Thr	Tyr	Asp	Lys	Gly	Phe	Thr	Gly	Ser	Ser	Val
1				5					10		

<210> 134

<211> 12

<212> PRT

<213> Homo sapiens

<400> 134

Gln	Ser	Tyr	Asp	Arg	Gly	Phe	Thr	Gly	Ala	Arg	Val
1				5					10		

<210> 135

<211> 12

<212> PRT

<213> Homo sapiens

<400> 135

Gln	Ser	Tyr	Glu	Arg	Gly	Phe	Thr	Gly	Ala	Arg	Val
1				5					10		

<210> 136

<211> 13

<212> PRT

<213> Homo sapiens

<400> 136

Gln	Ser	Tyr	Asp	Arg	Gly	Phe	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 137

- 43 -

<211> 13
<212> PRT
<213> Homo sapiens

<400> 137
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Phe Lys Val Phe
1 5 10

<210> 138
<211> 13
<212> PRT
<213> Homo sapiens

<400> 138
Gln Ser Tyr Asp Arg Gly Phe Val Ser Ala Tyr Val Phe
1 5 10

<210> 139
<211> 13
<212> PRT
<213> Homo sapiens

<400> 139
Gln Ser Tyr Asp Arg Gly Leu Thr Val Thr Lys Val Phe
1 5 10

<210> 140
<211> 13
<212> PRT
<213> Homo sapiens

<400> 140
Gln Ser Tyr Asp Arg Gly Tyr Thr Ala Ser Arg Val Phe
1 5 10

<210> 141
<211> 13
<212> PRT
<213> Homo sapiens

<400> 141
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Lys Val Phe
1 5 10

<210> 142
<211> 13
<212> PRT
<213> Homo sapiens

<400> 142
Gln Ser Tyr Asp Arg Gly Leu Thr Gly Phe Arg Val Phe
1 5 10

<210> 143
<211> 13
<212> PRT
<213> Homo sapiens

<400> 143
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Tyr Lys Val Phe

- 44 -

1 5 10

<210> 144

<211> 13

<212> PRT

<213> Homo sapiens

<400> 144

Gln Ser Tyr Asp Arg Gly Leu Thr Gly Tyr Arg Leu Phe
1 5 10

<210> 145

<211> 13

<212> PRT

<213> Homo sapiens

<400> 145

Gln Ser Tyr Asp Arg Gly Phe Thr Asp Tyr Lys Val Phe
1 5 10

<210> 146

<211> 13

<212> PRT

<213> Homo sapiens

<400> 146

Gln Ser Tyr Asp Arg Gly Phe Thr Gly Pro Arg Leu Phe
1 5 10

<210> 147

<211> 13

<212> PRT

<213> Homo sapiens

<400> 147

Gln Ser Tyr Asp Arg Gly Leu Thr Gly Ser Arg Val Phe
1 5 10

<210> 148

<211> 13

<212> PRT

<213> Homo sapiens

<400> 148

Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ala Arg Val Trp
1 5 10

<210> 149

<211> 13

<212> PRT

<213> Homo sapiens

<400> 149

Gln Ser Tyr Asp Arg Gly Phe Thr Gly Tyr Arg Val Phe
1 5 10

<210> 150

<211> 13

<212> PRT

- 45 -

<213> Homo sapiens

<400> 150

Gln	Ser	Tyr	Asp	Arg	Gly	Phe	Thr	Gly	Pro	Arg	Val	Phe
1				5					10			

<210> 151

<211> 13

<212> PRT

<213> Homo sapiens

<400> 151

Gln	Ser	Tyr	Asp	Arg	Gly	Met	Thr	Ser	Ser	Arg	Val	Phe
1				5					10			

<210> 152

<211> 13

<212> PRT

<213> Homo sapiens

<400> 152

Gln	Ser	Tyr	Asp	Arg	Asp	Ser	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 153

<211> 13

<212> PRT

<213> Homo sapiens

<400> 153

Gln	Ser	Tyr	Asp	Ser	Ser	Leu	Arg	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 154

<211> 13

<212> PRT

<213> Homo sapiens

<400> 154

His	Ser	Tyr	Asp	Ser	Asp	Phe	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 155

<211> 13

<212> PRT

<213> Homo sapiens

<400> 155

His	Ser	Ser	Glu	Ser	Gly	Phe	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 156

<211> 13

<212> PRT

<213> Homo sapiens

<400> 156

His	Ser	Tyr	Asp	Asn	Arg	Phe	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

- 46 -

<210> 157
<211> 13
<212> PRT
<213> Homo sapiens

<400> 157
His Ser Tyr Asp Ser Arg Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 158
<211> 13
<212> PRT
<213> Homo sapiens

<400> 158
Gln Ser Tyr Asp Ser Glu Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 159
<211> 13
<212> PRT
<213> Homo sapiens

<400> 159
Gln Ser Tyr Asp Thr Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 160
<211> 13
<212> PRT
<213> Homo sapiens

<400> 160
His Ser Tyr Asp Ser Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 161
<211> 13
<212> PRT
<213> Homo sapiens

<400> 161
Gln Ser Tyr Asp Thr Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 162
<211> 13
<212> PRT
<213> Homo sapiens

<400> 162
His Ser Tyr Asp Thr Lys Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 163
<211> 13
<212> PRT
<213> Homo sapiens

- 47 -

<400> 163

His Ser Ser Asp Ser Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 164

<211> 13

<212> PRT

<213> Homo sapiens

<400> 164

Gln Ser Tyr Asp Ser Asp Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 165

<211> 13

<212> PRT

<213> Homo sapiens

<400> 165

His Ser Tyr Glu Ser Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 166

<211> 13

<212> PRT

<213> Homo sapiens

<400> 166

Gln Ser Tyr Asp Ala Pro Trp Ser Gly Ser Arg Val Phe
1 5 10

<210> 167

<211> 13

<212> PRT

<213> Homo sapiens

<400> 167

Gln Ser Tyr Asp Ser Asp Phe Thr Gly Ser Lys Val Phe
1 5 10

<210> 168

<211> 13

<212> PRT

<213> Homo sapiens

<400> 168

His Thr Asn Asp Ser Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 169

<211> 13

<212> PRT

<213> Homo sapiens

<400> 169

His Ser Tyr Asp Thr Arg Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 170

- 48 -

<211> 13
<212> PRT
<213> Homo sapiens

<400> 170
Gln Ser Tyr Asp Met Arg Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 171
<211> 13
<212> PRT
<213> Homo sapiens

<400> 171
His Ser Ser Asp Ser Asp Ser Thr Gly Ser Arg Val Phe
1 5 10

<210> 172
<211> 13
<212> PRT
<213> Homo sapiens

<400> 172
Gln Ser Tyr Asn Thr Asp Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 173
<211> 13
<212> PRT
<213> Homo sapiens

<400> 173
Gln Ser Tyr Asp Ser Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 174
<211> 13
<212> PRT
<213> Homo sapiens

<400> 174
His Ser Tyr Asp Met Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 175
<211> 13
<212> PRT
<213> Homo sapiens

<400> 175
His Ser Tyr Asp Asn Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 176
<211> 13
<212> PRT
<213> Homo sapiens

<400> 176
His Ser His Asp Arg Asp Phe Thr Gly Ser Arg Val Phe

- 49 -

1 5 10

<210> 177
<211> 12
<212> PRT
<213> Homo sapiens

<400> 177
Gln Ser Tyr Asp Ser Ser Leu Arg Gly Ser Arg Val
1 5 10

<210> 178
<211> 13
<212> PRT
<213> Homo sapiens

<400> 178
Gln Ser Tyr Asp Arg Gly Ile His Gly Ser Arg Val Phe
1 5 10

<210> 179
<211> 13
<212> PRT
<213> Homo sapiens

<400> 179
Gln Ser Tyr Asp Ser Gly Phe Pro Gly Ser Arg Val Phe
1 5 10

<210> 180
<211> 13
<212> PRT
<213> Homo sapiens

<400> 180
Gln Ser Tyr Asp Ile Gly Ser Thr Gly Ser Arg Val Phe
1 5 10

<210> 181
<211> 13
<212> PRT
<213> Homo sapiens

<400> 181
Gln Ser Tyr Asp Ser Gly Leu Thr Gly Ser Arg Val Phe
1 5 10

<210> 182
<211> 13
<212> PRT
<213> Homo sapiens

<400> 182
Gln Ser Tyr Asp Ile Gly Met Thr Gly Ser Arg Val Phe
1 5 10

<210> 183
<211> 13
<212> PRT

- 50 -

<213> Homo sapiens

<400> 183

Gln	Ser	Tyr	Asp	Ile	Gly	Leu	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 184

<211> 13

<212> PRT

<213> Homo sapiens

<400> 184

Gln	Ser	Tyr	Asp	Ser	Gly	Val	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 185

<211> 13

<212> PRT

<213> Homo sapiens

<400> 185

Gln	Ser	Tyr	Asp	Arg	Gly	Leu	Thr	Ala	Ser	Arg	Val	Phe
1				5					10			

<210> 186

<211> 13

<212> PRT

<213> Homo sapiens

<400> 186

Gln	Ser	Tyr	Asp	Thr	Gly	Leu	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 187

<211> 13

<212> PRT

<213> Homo sapiens

<400> 187

Gln	Ser	Tyr	Asp	Thr	Ala	Leu	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 188

<211> 13

<212> PRT

<213> Homo sapiens

<400> 188

Gln	Ser	Tyr	Asp	Ile	Arg	Phe	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

<210> 189

<211> 13

<212> PRT

<213> Homo sapiens

<400> 189

Gln	Ser	Tyr	Asp	Ile	Arg	Ser	Thr	Gly	Ser	Arg	Val	Phe
1				5					10			

- 51 -

<210> 190
<211> 13
<212> PRT
<213> Homo sapiens

<400> 190
Gln Ser Tyr Asp Asn Arg Leu Thr Gly Ser Arg Val Phe
1 5 10

<210> 191
<211> 13
<212> PRT
<213> Homo sapiens

<400> 191
Gln Ser Tyr Glu Thr Ser Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 192
<211> 13
<212> PRT
<213> Homo sapiens

<400> 192
Gln Ser Tyr Asp Ser Ser Ser Thr Gly Ser Arg Val Phe
1 5 10

<210> 193
<211> 13
<212> PRT
<213> Homo sapiens

<400> 193
Gln Ser Tyr Asp Ser Gly Phe Thr Ala Ser Arg Val Phe
1 5 10

<210> 194
<211> 13
<212> PRT
<213> Homo sapiens

<400> 194
Gln Thr Tyr Asp Lys Gly Phe Thr Gly Ser Ser Val Phe
1 5 10

<210> 195
<211> 13
<212> PRT
<213> Homo sapiens

<400> 195
Gln Ser Tyr Asp Asn Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 196
<211> 13
<212> PRT
<213> Homo sapiens

- 52 -

<400> 196
Gln Ser Tyr Asp Thr Gly Phe Thr Lys Ser Arg Val Phe
1 5 10

<210> 197
<211> 13
<212> PRT
<213> Homo sapiens

<400> 197
Gln Ser Tyr Asp Ser Asp Val Thr Gly Ser Arg Val Phe
1 5 10

<210> 198
<211> 13
<212> PRT
<213> Homo sapiens

<400> 198
Gln Ser Tyr Asp Ala Gly Phe Thr Gly Ser Arg Val Phe
1 5 10

<210> 199
<211> 12
<212> PRT
<213> Homo sapiens

<400> 199
Gln Ser Tyr Asp Arg Gly Thr His Pro Ser Met Leu
1 5 10

<210> 200
<211> 12
<212> PRT
<213> Homo sapiens

<400> 200
Gln Ser Tyr Asp Arg Gly Thr Thr Pro Arg Pro Met
1 5 10

<210> 201
<211> 12
<212> PRT
<213> Homo sapiens

<400> 201
Gln Ser Tyr Asp Arg Gly Arg Asn Pro Ala Leu Thr
1 5 10

<210> 202
<211> 12
<212> PRT
<213> Homo sapiens

<400> 202
Gln Ser Tyr Asp Arg Gly Thr His Pro Trp Leu His
1 5 10

<210> 203

- 53 -

<211> 12
<212> PRT
<213> Homo sapiens

<400> 203
Gln Ser Tyr Asp Arg Gly Asn Ser Pro Ala Thr Val
1 5 10

<210> 204
<211> 12
<212> PRT
<213> Homo sapiens

<400> 204
Gln Ser Tyr Asp Arg Gly Thr Phe Pro Ser Pro Gln
1 5 10

<210> 205
<211> 12
<212> PRT
<213> Homo sapiens

<400> 205
Gln Ser Tyr Asp Arg Gly Leu Asn Pro Ser Ala Thr
1 5 10

<210> 206
<211> 12
<212> PRT
<213> Homo sapiens

<400> 206
Gln Ser Tyr Asp Arg Gly Lys Ser Asn Lys Met Leu
1 5 10

<210> 207
<211> 12
<212> PRT
<213> Homo sapiens

<400> 207
Gln Ser Tyr Asp Arg Gly His Thr Ala His Leu Tyr
1 5 10

<210> 208
<211> 12
<212> PRT
<213> Homo sapiens

<400> 208
Gln Ser Tyr Asp Arg Gly Gln Thr Pro Ser Ile Thr
1 5 10

<210> 209
<211> 12
<212> PRT
<213> Homo sapiens

<400> 209
Gln Ser Tyr Asp Arg Gly Tyr Pro Arg Asn Ile Leu

- 54 -

1 5 10

<210> 210
<211> 12
<212> PRT
<213> Homo sapiens

<400> 210
Gln Ser Tyr Asp Arg Gly Ile Thr Pro Gly Leu Ala
1 5 10

<210> 211
<211> 12
<212> PRT
<213> Homo sapiens

<400> 211
Gln Ser Tyr Asp Arg Gly Gln Pro His Ala Val Leu
1 5 10

<210> 212
<211> 12
<212> PRT
<213> Homo sapiens

<400> 212
Gln Ser Tyr Asp Arg Gly Asn Ser Pro Ile Pro Thr
1 5 10

<210> 213
<211> 12
<212> PRT
<213> Homo sapiens

<400> 213
Gln Ser Tyr Asp Arg Gly Thr Pro Asn Asn Ser Phe
1 5 10

<210> 214
<211> 12
<212> PRT
<213> Homo sapiens

<400> 214
Gln Ser Tyr Asp Ser Gly Val Asp Pro Gly Pro Tyr
1 5 10

<210> 215
<211> 12
<212> PRT
<213> Homo sapiens

<400> 215
Gln Ser Tyr Asp Arg Gly Arg Pro Arg His Ala Leu
1 5 10

<210> 216
<211> 12
<212> PRT

- 55 -

<213> Homo sapiens

<400> 216

Gln Ser Tyr Asp Arg Gly Pro Tyr His Pro Ile Arg
1 5 10

<210> 217

<211> 12

<212> PRT

<213> Homo sapiens

<400> 217

Gln Ser Tyr Asp Arg Gly Pro His Thr Gln Pro Thr
1 5 10

<210> 218

<211> 12

<212> PRT

<213> Homo sapiens

<400> 218

Gln Ser Tyr Asp Arg Gly His Asn Asn Phe Ser Pro
1 5 10

<210> 219

<211> 12

<212> PRT

<213> Homo sapiens

<400> 219

Gln Ser Tyr Asp Arg Gly Pro Thr His Leu Pro His
1 5 10

<210> 220

<211> 12

<212> PRT

<213> Homo sapiens

<400> 220

Gln Ser Tyr Asp Arg Gly Thr Pro Ser Tyr Pro Thr
1 5 10

<210> 221

<211> 12

<212> PRT

<213> Homo sapiens

<400> 221

Gln Ser Tyr Asp Ser Gly Thr Ser Asn Leu Leu Pro
1 5 10

<210> 222

<211> 12

<212> PRT

<213> Homo sapiens

<400> 222

Gln Ser Tyr Asp Arg Gly Asp Ser Asn His Asp Leu
1 5 10

- 56 -

<210> 223
<211> 12
<212> PRT
<213> Homo sapiens

<400> 223
Gln Ser Tyr Asp Arg Gly Leu Pro Arg Leu Thr His
1 5 10

<210> 224
<211> 12
<212> PRT
<213> Homo sapiens

<400> 224
Gln Ser Tyr Asp Arg Gly Ile Pro Thr Ser Tyr Leu
1 5 10

<210> 225
<211> 12
<212> PRT
<213> Homo sapiens

<400> 225
Gln Ser Tyr Asp Arg Gly Leu Arg Val Gln Ala Pro
1 5 10

<210> 226
<211> 12
<212> PRT
<213> Homo sapiens

<400> 226
Gln Ser Tyr Asp Arg Gly Leu Ser Asp Ser Pro Leu
1 5 10

<210> 227
<211> 12
<212> PRT
<213> Homo sapiens

<400> 227
Gln Ser Tyr Asp Ser Gly Ser Leu Arg Arg Ile Leu
1 5 10

<210> 228
<211> 12
<212> PRT
<213> Homo sapiens

<400> 228
Gln Ser Tyr Asp Arg Gly Pro Ala Arg Thr Ser Pro
1 5 10

<210> 229
<211> 12
<212> PRT
<213> Homo sapiens

- 57 -

<400> 229

Gln Ser Tyr Asp Arg Gly Arg Ala Ala His Pro Gln
1 5 10

<210> 230

<211> 12

<212> PRT

<213> Homo sapiens

<400> 230

Gln Ser Tyr Asp Arg Gly Thr Gln Pro Ala Asx Ile
1 5 10

<210> 231

<211> 12

<212> PRT

<213> Homo sapiens

<400> 231

Gln Ser Tyr Asp Arg Gly Thr His Pro Thr Met Ile
1 5 10

<210> 232

<211> 12

<212> PRT

<213> Homo sapiens

<400> 232

Gln Ser Tyr Asp Arg Gly Arg Ile Pro Ala Asx Thr
1 5 10

<210> 233

<211> 12

<212> PRT

<213> Homo sapiens

<400> 233

Gln Ser Tyr Asp Arg Gly Thr His Pro Val Pro Ala
1 5 10

<210> 234

<211> 12

<212> PRT

<213> Homo sapiens

<400> 234

Gln Ser Tyr Asp Arg Gly Ser Asx Pro Ile Pro Ala
1 5 10

<210> 235

<211> 12

<212> PRT

<213> Homo sapiens

<400> 235

Gln Ser Tyr Asp Arg Gly Thr His Pro Val Pro Ala
1 5 10

<210> 236

- 58 -

<211> 12
<212> PRT
<213> Homo sapiens

<400> 236
Gln Ser Tyr Asp Arg Gly Thr His Pro Thr Met Tyr
1 5 10

<210> 237
<211> 12
<212> PRT
<213> Homo sapiens

<400> 237
Gln Ser Tyr Asp Arg Gly His His Tyr Thr Thr Phe
1 5 10

<210> 238
<211> 12
<212> PRT
<213> Homo sapiens

<400> 238
Gln Ser Tyr Asp Arg Gly Ser His Pro Ala Ala Glu
1 5 10

<210> 239
<211> 12
<212> PRT
<213> Homo sapiens

<400> 239
Gln Ser Tyr Asp Arg Gly Thr Ile Pro Ser Ile Glu
1 5 10

<210> 240
<211> 12
<212> PRT
<213> Homo sapiens

<400> 240
Gln Ser Tyr Asp Arg Gly Ser Ser Pro Ala Ile Met
1 5 10

<210> 241
<211> 12
<212> PRT
<213> Homo sapiens

<400> 241
Gln Ser Tyr Asp Arg Gly Ile Trp Pro Asn Leu Asn
1 5 10

<210> 242
<211> 12
<212> PRT
<213> Homo sapiens

<400> 242
Gln Ser Tyr Asp Arg Gly Thr His Pro Asn Leu Asn

- 59 -

1 5 10

<210> 243
<211> 12
<212> PRT
<213> Homo sapiens

<400> 243
Gln Ser Tyr Asp Arg Gly Thr His Pro Ser Ile Ser
1 5 10

<210> 244
<211> 12
<212> PRT
<213> Homo sapiens

<400> 244
Gln Ser Tyr Asp Arg Gly Ser Ala Pro Met Ile Asn
1 5 10

<210> 245
<211> 12
<212> PRT
<213> Homo sapiens

<400> 245
Gln Ser Tyr Asp Arg Gly His His Pro Ala Met Ser
1 5 10

<210> 246
<211> 12
<212> PRT
<213> Homo sapiens

<400> 246
Gln Ser Tyr Asp Arg Gly Thr His Pro Ser Ile Thr
1 5 10

<210> 247
<211> 12
<212> PRT
<213> Homo sapiens

<400> 247
Gln Ser Tyr Asp Arg Gly Thr Asp Pro Ala Ile Val
1 5 10

<210> 248
<211> 12
<212> PRT
<213> Homo sapiens

<400> 248
Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 249
<211> 12
<212> PRT

- 60 -

<213> Homo sapiens

<400> 249

Gln	Ser	Tyr	Asp	Arg	Gly	Ser	His	Pro	Ala	Leu	Thr
1				5					10		

<210> 250

<211> 12

<212> PRT

<213> Homo sapiens

<400> 250

Gln	Ser	Tyr	Asp	Arg	Gly	Thr	Thr	Pro	Ala	Pro	Glu
1				5					10		

<210> 251

<211> 12

<212> PRT

<213> Homo sapiens

<400> 251

Gln	Ser	Tyr	Asp	Arg	Gly	Ser	His	Pro	Thr	Leu	Ile
1				5					10		

<210> 252

<211> 12

<212> PRT

<213> Homo sapiens

<400> 252

Gln	Ser	Tyr	Asp	Arg	Gly	Thr	His	Pro	Ser	Met	Leu
1				5					10		

<210> 253

<211> 12

<212> PRT

<213> Homo sapiens

<400> 253

Gln	Ser	Tyr	Asp	Arg	Gly	Thr	Thr	Pro	Arg	Pro	Met
1				5					10		

<210> 254

<211> 12

<212> PRT

<213> Homo sapiens

<400> 254

Gln	Ser	Tyr	Asp	Arg	Gly	Arg	Leu	Pro	Ala	Gln	Thr
1				5					10		

<210> 255

<211> 12

<212> PRT

<213> Homo sapiens

<400> 255

Gln	Ser	Tyr	Asp	Arg	Gly	Thr	His	Pro	Leu	Thr	Ile
1				5					10		

- 61 -

<210> 256
<211> 12
<212> PRT
<213> Homo sapiens

<400> 256
Gln Ser Tyr Asp Arg Gly Gln Thr Pro Ser Ile Thr
1 5 10

<210> 257
<211> 12
<212> PRT
<213> Homo sapiens

<400> 257
Gln Ser Tyr Asp Arg Gly Thr His Phe Gln Met Tyr
1 5 10

<210> 258
<211> 12
<212> PRT
<213> Homo sapiens

<400> 258
Gln Ser Tyr Asp Arg Gly Arg Asn Pro Ala Leu Thr
1 5 10

<210> 259
<211> 12
<212> PRT
<213> Homo sapiens

<400> 259
Gln Ser Tyr Asp Arg Gly Thr His Pro Leu Thr Met
1 5 10

<210> 260
<211> 12
<212> PRT
<213> Homo sapiens

<400> 260
Gln Ser Tyr Asp Arg Gly Thr His Pro Leu Thr Met
1 5 10

<210> 261
<211> 12
<212> PRT
<213> Homo sapiens

<400> 261
Gln Ser Tyr Asp Ser Gly Tyr Thr Gly Ser Arg Val
1 5 10

<210> 262
<211> 12
<212> PRT
<213> Homo sapiens

- 62 -

<400> 262
Gln Ser Tyr Asp Ser Gly Phe Thr Gly Ser Arg Val
1 5 10

<210> 263
<211> 12
<212> PRT
<213> Homo sapiens

<400> 263
Gln Ser Tyr Asp Ser Arg Phe Thr Gly Ser Arg Val
1 5 10

<210> 264
<211> 12
<212> PRT
<213> Homo sapiens

<400> 264
Gln Ser Tyr Pro Asp Gly Thr Pro Ala Ser Arg Val
1 5 10

<210> 265
<211> 12
<212> PRT
<213> Homo sapiens

<400> 265
Gln Ser Tyr Ser Thr His Met Pro Ile Ser Arg Val
1 5 10

<210> 266
<211> 12
<212> PRT
<213> Homo sapiens

<400> 266
Gln Ser Tyr Asp Ser Gly Ser Thr Gly Ser Arg Val
1 5 10

<210> 267
<211> 12
<212> PRT
<213> Homo sapiens

<400> 267
Gln Ser Tyr Pro Asn Ser Tyr Pro Ile Ser Arg Val
1 5 10

<210> 268
<211> 10
<212> PRT
<213> Homo sapiens

<400> 268
Gln Ser Tyr Ile Arg Ala Pro Gln Gln Val
1 5 10

<210> 269

- 63 -

<211> 12
<212> PRT
<213> Homo sapiens

<400> 269
Gln Ser Tyr Leu Lys Ser Arg Ala Phe Ser Arg Val
1 5 10

<210> 270
<211> 12
<212> PRT
<213> Homo sapiens

<400> 270
Gln Ser Tyr Asp Ser Arg Phe Thr Gly Ser Arg Val
1 5 10

<210> 271
<211> 12
<212> PRT
<213> Homo sapiens

<400> 271
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 272
<211> 12
<212> PRT
<213> Homo sapiens

<400> 272
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 273
<211> 12
<212> PRT
<213> Homo sapiens

<400> 273
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Phe Asp Gly
1 5 10

<210> 274
<211> 12
<212> PRT
<213> Homo sapiens

<400> 274
Gln Ser Tyr Asp Arg Gly Thr Ala Pro Ala Leu Ser
1 5 10

<210> 275
<211> 12
<212> PRT
<213> Homo sapiens

<400> 275
Gln Ser Tyr Asp Arg Gly Ser Tyr Pro Ala Leu Arg

- 64 -

1 5 10

<210> 276
<211> 12
<212> PRT
<213> Homo sapiens

<400> 276
Gln Ser Tyr Asp Arg Gly Asn Trp Pro Asn Ser Asn
1 5 10

<210> 277
<211> 12
<212> PRT
<213> Homo sapiens

<400> 277
Gln Ser Tyr Asp Arg Gly Thr Ala Pro Ser Leu Leu
1 5 10

<210> 278
<211> 12
<212> PRT
<213> Homo sapiens

<400> 278
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 279
<211> 12
<212> PRT
<213> Homo sapiens

<400> 279
Gln Ser Tyr Asp Arg Gly Thr Thr Pro Arg Ile Arg
1 5 10

<210> 280
<211> 12
<212> PRT
<213> Homo sapiens

<400> 280
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 281
<211> 12
<212> PRT
<213> Homo sapiens

<400> 281
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 282
<211> 12
<212> PRT

- 65 -

<213> Homo sapiens

<400> 282

Gln Ser Tyr Asp Arg Gly Met Ile Pro Ala Leu Thr
1 5 10

<210> 283

<211> 12

<212> PRT

<213> Homo sapiens

<400> 283

Gln Ser Tyr Asp Arg Asn Thr His Pro Ala Leu Leu
1 5 10

<210> 284

<211> 12

<212> PRT

<213> Homo sapiens

<400> 284

Gln Ser Tyr Asp Arg Phe Thr His Pro Ala Leu Leu
1 5 10

<210> 285

<211> 12

<212> PRT

<213> Homo sapiens

<400> 285

Gln Ser Tyr Asp Arg Tyr Thr His Pro Ala Leu Leu
1 5 10

<210> 286

<211> 12

<212> PRT

<213> Homo sapiens

<400> 286

Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 287

<211> 12

<212> PRT

<213> Homo sapiens

<400> 287

Gln Ser Tyr Asp Arg Tyr Thr His Pro Ala Leu Leu
1 5 10

<210> 288

<211> 9

<212> PRT

<213> Homo sapiens

<400> 288

Phe Thr Phe Glu Ser Tyr Gly Met His
1 5

- 66 -

<210> 289
<211> 9
<212> PRT
<213> Homo sapiens

<400> 289
Phe Thr Phe Ser Ser Tyr Gly Met His
1 5

<210> 290
<211> 9
<212> PRT
<213> Homo sapiens

<400> 290
Phe Thr Phe Tyr Ser Tyr Gly Met His
1 5

<210> 291
<211> 9
<212> PRT
<213> Homo sapiens

<400> 291
Phe Thr Phe His Ser Tyr Gly Met His
1 5

<210> 292
<211> 9
<212> PRT
<213> Homo sapiens

<400> 292
Phe Thr Phe Lys Ser Tyr Gly Met His
1 5

<210> 293
<211> 9
<212> PRT
<213> Homo sapiens

<400> 293
Phe Thr Phe Arg Ser Tyr Gly Met His
1 5

<210> 294
<211> 9
<212> PRT
<213> Homo sapiens

<400> 294
Phe Thr Phe Asn Ser Tyr Gly Met His
1 5

<210> 295
<211> 9
<212> PRT
<213> Homo sapiens

- 67 -

<400> 295
Phe Thr Phe Thr Ser Tyr Gly Met His
1 5

<210> 296
<211> 9
<212> PRT
<213> Homo sapiens

<400> 296
Phe Thr Phe Gly Ser Tyr Gly Met His
1 5

<210> 297
<211> 9
<212> PRT
<213> Homo sapiens

<400> 297
Phe Thr Phe Val Ser Tyr Gly Met His
1 5

<210> 298
<211> 9
<212> PRT
<213> Homo sapiens

<400> 298
Phe Thr Phe Ile Ser Tyr Gly Met His
1 5

<210> 299
<211> 9
<212> PRT
<213> Homo sapiens

<400> 299
Phe Thr Phe Trp Ser Tyr Gly Met His
1 5

<210> 300
<211> 9
<212> PRT
<213> Homo sapiens

<400> 300
Phe Thr Phe Ser Glu Tyr Gly Met His
1 5

<210> 301
<211> 9
<212> PRT
<213> Homo sapiens

<400> 301
Phe Thr Phe Ser Cys Tyr Gly Met His
1 5

<210> 302

- 68 -

<211> 9
<212> PRT
<213> Homo sapiens

<400> 302
Phe Thr Phe Ser Ser Tyr Gly Met His
1 5

<210> 303
<211> 9
<212> PRT
<213> Homo sapiens

<400> 303
Phe Thr Phe Ser Tyr Tyr Gly Met His
1 5

<210> 304
<211> 9
<212> PRT
<213> Homo sapiens

<400> 304
Phe Thr Phe Ser His Tyr Gly Met His
1 5

<210> 305
<211> 9
<212> PRT
<213> Homo sapiens

<400> 305
Phe Thr Phe Ser Arg Tyr Gly Met His
1 5

<210> 306
<211> 9
<212> PRT
<213> Homo sapiens

<400> 306
Phe Thr Phe Ser Asn Tyr Gly Met His
1 5

<210> 307
<211> 9
<212> PRT
<213> Homo sapiens

<400> 307
Phe Thr Phe Ser Gln Tyr Gly Met His
1 5

<210> 308
<211> 9
<212> PRT
<213> Homo sapiens

<400> 308
Phe Thr Phe Ser Thr Tyr Gly Met His

- 69 -

1 5

<210> 309
<211> 9
<212> PRT
<213> Homo sapiens

<400> 309
Phe Thr Phe Ser Ala Tyr Gly Met His
1 5

<210> 310
<211> 9
<212> PRT
<213> Homo sapiens

<400> 310
Phe Thr Phe Ser Ile Tyr Gly Met His
1 5

<210> 311
<211> 9
<212> PRT
<213> Homo sapiens

<400> 311
Phe Thr Phe Ser Ser Glu Gly Met His
1 5

<210> 312
<211> 9
<212> PRT
<213> Homo sapiens

<400> 312
Phe Thr Phe Ser Ser Cys Gly Met His
1 5

<210> 313
<211> 9
<212> PRT
<213> Homo sapiens

<400> 313
Phe Thr Phe Ser Ser Ser Gly Met His
1 5

<210> 314
<211> 9
<212> PRT
<213> Homo sapiens

<400> 314
Phe Thr Phe Ser Ser Tyr Gly Met His
1 5

<210> 315
<211> 9
<212> PRT

- 70 -

<213> Homo sapiens

<400> 315

Phe Thr Phe Ser Ser His Gly Met His
1 5

<210> 316

<211> 9

<212> PRT

<213> Homo sapiens

<400> 316

Phe Thr Phe Ser Ser Arg Gly Met His
1 5

<210> 317

<211> 9

<212> PRT

<213> Homo sapiens

<400> 317

Phe Thr Phe Ser Ser Asn Gly Met His
1 5

<210> 318

<211> 9

<212> PRT

<213> Homo sapiens

<400> 318

Phe Thr Phe Ser Ser Thr Gly Met His
1 5

<210> 319

<211> 9

<212> PRT

<213> Homo sapiens

<400> 319

Phe Thr Phe Ser Ser Ala Gly Met His
1 5

<210> 320

<211> 9

<212> PRT

<213> Homo sapiens

<400> 320

Phe Thr Phe Ser Ser Val Gly Met His
1 5

<210> 321

<211> 9

<212> PRT

<213> Homo sapiens

<400> 321

Phe Thr Phe Ser Ser Leu Gly Met His
1 5

- 71 -

<210> 322
<211> 9
<212> PRT
<213> Homo sapiens

<400> 322
Phe Thr Phe Ser Ser Ile Gly Met His
1 5

<210> 323
<211> 9
<212> PRT
<213> Homo sapiens

<400> 323
Phe Thr Phe Ser Ser Tyr Asp Met His
1 5

<210> 324
<211> 9
<212> PRT
<213> Homo sapiens

<400> 324
Phe Thr Phe Ser Ser Tyr Glu Met His
1 5

<210> 325
<211> 9
<212> PRT
<213> Homo sapiens

<400> 325
Phe Thr Phe Ser Ser Tyr Cys Met His
1 5

<210> 326
<211> 9
<212> PRT
<213> Homo sapiens

<400> 326
Phe Thr Phe Ser Ser Tyr Ser Met His
1 5

<210> 327
<211> 9
<212> PRT
<213> Homo sapiens

<400> 327
Phe Thr Phe Ser Ser Tyr Tyr Met His
1 5

<210> 328
<211> 9
<212> PRT
<213> Homo sapiens

- 72 -

<400> 328
Phe Thr Phe Ser Ser Tyr Asn Met His
1 5

<210> 329
<211> 9
<212> PRT
<213> Homo sapiens

<400> 329
Phe Thr Phe Ser Ser Tyr Gly Met His
1 5

<210> 330
<211> 9
<212> PRT
<213> Homo sapiens

<400> 330
Phe Thr Phe Ser Ser Tyr Ala Met His
1 5

<210> 331
<211> 9
<212> PRT
<213> Homo sapiens

<400> 331
Phe Thr Phe Ser Ser Tyr Val Met His
1 5

<210> 332
<211> 9
<212> PRT
<213> Homo sapiens

<400> 332
Phe Thr Phe Ser Ser Tyr Met Met His
1 5

<210> 333
<211> 9
<212> PRT
<213> Homo sapiens

<400> 333
Phe Thr Phe Ser Ser Tyr Ile Met His
1 5

<210> 334
<211> 9
<212> PRT
<213> Homo sapiens

<400> 334
Phe Thr Phe Ser Ser Tyr Pro Met His
1 5

<210> 335

- 73 -

<211> 17
<212> PRT
<213> Homo sapiens

<400> 335
Glu Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 336
<211> 17
<212> PRT
<213> Homo sapiens

<400> 336
Cys Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 337
<211> 17
<212> PRT
<213> Homo sapiens

<400> 337
Tyr Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 338
<211> 17
<212> PRT
<213> Homo sapiens

<400> 338
His Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 339
<211> 17
<212> PRT
<213> Homo sapiens

<400> 339
Lys Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 340
<211> 17

- 74 -

<212> PRT

<213> Homo sapiens

<400> 340

Asn	Ile	Arg	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 341

<211> 17

<212> PRT

<213> Homo sapiens

<400> 341

Gln	Ile	Arg	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 342

<211> 17

<212> PRT

<213> Homo sapiens

<400> 342

Thr	Ile	Arg	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 343

<211> 17

<212> PRT

<213> Homo sapiens

<400> 343

Leu	Ile	Arg	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 344

<211> 17

<212> PRT

<213> Homo sapiens

<400> 344

Phe	Ile	Arg	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 345

<211> 17

<212> PRT

- 75 -

<213> Homo sapiens

<400> 345

Phe	Ile	Glu	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5				10						15	

Gly

<210> 346

<211> 17

<212> PRT

<213> Homo sapiens

<400> 346

Phe	Ile	Ser	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5				10						15	

Gly

<210> 347

<211> 17

<212> PRT

<213> Homo sapiens

<400> 347

Phe	Ile	Tyr	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5				10						15	

Gly

<210> 348

<211> 17

<212> PRT

<213> Homo sapiens

<400> 348

Phe	Ile	His	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5				10						15	

Gly

<210> 349

<211> 17

<212> PRT

<213> Homo sapiens

<400> 349

Phe	Ile	Lys	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5				10						15	

Gly

<210> 350

<211> 17

<212> PRT

<213> Homo sapiens

- 76 -

<400> 350

Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 351

<211> 17

<212> PRT

<213> Homo sapiens

<400> 351

Phe Ile Gln Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 352

<211> 17

<212> PRT

<213> Homo sapiens

<400> 352

Phe Ile Thr Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 353

<211> 17

<212> PRT

<213> Homo sapiens

<400> 353

Phe Ile Gly Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 354

<211> 17

<212> PRT

<213> Homo sapiens

<400> 354

Phe Ile Ala Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 355

<211> 17

<212> PRT

<213> Homo sapiens

- 77 -

<400> 355

Phe Ile Val Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 356

<211> 17

<212> PRT

<213> Homo sapiens

<400> 356

Phe Ile Leu Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 357

<211> 17

<212> PRT

<213> Homo sapiens

<400> 357

Phe Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 358

<211> 17

<212> PRT

<213> Homo sapiens

<400> 358

Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 359

<211> 17

<212> PRT

<213> Homo sapiens

<400> 359

Phe Ile Arg Tyr Glu Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 360

<211> 17

<212> PRT

<213> Homo sapiens

<400> 360

- 78 -

Phe Ile Arg Tyr Ser Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 361

<211> 17

<212> PRT

<213> Homo sapiens

<400> 361

Phe Ile Arg Tyr Tyr Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 362

<211> 17

<212> PRT

<213> Homo sapiens

<400> 362

Phe Ile Arg Tyr Lys Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 363

<211> 17

<212> PRT

<213> Homo sapiens

<400> 363

Phe Ile Arg Tyr Arg Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 364

<211> 17

<212> PRT

<213> Homo sapiens

<400> 364

Phe Ile Arg Tyr Asn Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 365

<211> 17

<212> PRT

<213> Homo sapiens

<400> 365

Phe Ile Arg Tyr Gln Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys

- 79 -

1 5 10 15
Gly

<210> 366
<211> 17
<212> PRT
<213> Homo sapiens

<400> 366
Phe Ile Arg Tyr Thr Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 367
<211> 17
<212> PRT
<213> Homo sapiens

<400> 367
Phe Ile Arg Tyr Ala Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 368
<211> 17
<212> PRT
<213> Homo sapiens

<400> 368
Phe Ile Arg Tyr Val Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 369
<211> 17
<212> PRT
<213> Homo sapiens

<400> 369
Phe Ile Arg Tyr Leu Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 370
<211> 17
<212> PRT
<213> Homo sapiens

<400> 370
Phe Ile Arg Tyr Ile Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

- 80 -

Gly

<210> 371
<211> 17
<212> PRT
<213> Homo sapiens

<400> 371
Phe Ile Arg Tyr Phe Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 372
<211> 17
<212> PRT
<213> Homo sapiens

<400> 372
Phe Ile Arg Tyr Asp Asp Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 373
<211> 17
<212> PRT
<213> Homo sapiens

<400> 373
Phe Ile Arg Tyr Asp Glu Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 374
<211> 17
<212> PRT
<213> Homo sapiens

<400> 374
Phe Ile Arg Tyr Asp Ser Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 375
<211> 17
<212> PRT
<213> Homo sapiens

<400> 375
Phe Ile Arg Tyr Asp Tyr Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

- 81 -

Gly

<210> 376
<211> 17
<212> PRT
<213> Homo sapiens

<400> 376
Phe Ile Arg Tyr Asp Lys Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 377
<211> 17
<212> PRT
<213> Homo sapiens

<400> 377
Phe Ile Arg Tyr Asp Arg Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 378
<211> 17
<212> PRT
<213> Homo sapiens

<400> 378
Phe Ile Arg Tyr Asp Asn Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 379
<211> 17
<212> PRT
<213> Homo sapiens

<400> 379
Phe Ile Arg Tyr Asp Gln Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 380
<211> 17
<212> PRT
<213> Homo sapiens

<400> 380
Phe Ile Arg Tyr Asp Thr Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

- 82 -

<210> 381
<211> 17
<212> PRT
<213> Homo sapiens

<400> 381
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 382
<211> 17
<212> PRT
<213> Homo sapiens

<400> 382
Phe Ile Arg Tyr Asp Val Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 383
<211> 17
<212> PRT
<213> Homo sapiens

<400> 383
Phe Ile Arg Tyr Asp Phe Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 384
<211> 17
<212> PRT
<213> Homo sapiens

<400> 384
Phe Ile Arg Tyr Asp Gly Ser Ser Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 385
<211> 17
<212> PRT
<213> Homo sapiens

<400> 385
Phe Ile Arg Tyr Asp Gly Ser Tyr Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

- 83 -

<210> 386
<211> 17
<212> PRT
<213> Homo sapiens

<400> 386
Phe Ile Arg Tyr Asp Gly Ser His Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 387
<211> 17
<212> PRT
<213> Homo sapiens

<400> 387
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 388
<211> 17
<212> PRT
<213> Homo sapiens

<400> 388
Phe Ile Arg Tyr Asp Gly Ser Thr Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 389
<211> 17
<212> PRT
<213> Homo sapiens

<400> 389
Phe Ile Arg Tyr Asp Gly Ser Gly Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 390
<211> 17
<212> PRT
<213> Homo sapiens

<400> 390
Phe Ile Arg Tyr Asp Gly Ser Met Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

- 84 -

<210> 391
<211> 17
<212> PRT
<213> Homo sapiens

<400> 391
Phe Ile Arg Tyr Asp Gly Ser Leu Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 392
<211> 17
<212> PRT
<213> Homo sapiens

<400> 392
Phe Ile Arg Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 393
<211> 17
<212> PRT
<213> Homo sapiens

<400> 393
Phe Ile Arg Tyr Asp Gly Ser Pro Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 394
<211> 17
<212> PRT
<213> Homo sapiens

<400> 394
Phe Ile Arg Tyr Asp Gly Ser Phe Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 395
<211> 17
<212> PRT
<213> Homo sapiens

<400> 395
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Glu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

- 85 -

<210> 396
<211> 17
<212> PRT
<213> Homo sapiens

<400> 396
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Ser Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 397
<211> 17
<212> PRT
<213> Homo sapiens

<400> 397
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 398
<211> 17
<212> PRT
<213> Homo sapiens

<400> 398
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 399
<211> 17
<212> PRT
<213> Homo sapiens

<400> 399
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Val Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 400
<211> 17
<212> PRT
<213> Homo sapiens

<400> 400
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 401

- 86 -

<211> 17
<212> PRT
<213> Homo sapiens

<400> 401
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Ile Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 402
<211> 17
<212> PRT
<213> Homo sapiens

<400> 402
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Pro Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 403
<211> 17
<212> PRT
<213> Homo sapiens

<400> 403
Phe Ile Arg Tyr Asp Gly Ser Asn Lys Phe Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 404
<211> 6
<212> PRT
<213> Homo sapiens

<400> 404
Glu Gly Ser His Asp Asn
1 5

<210> 405
<211> 6
<212> PRT
<213> Homo sapiens

<400> 405
Ser Gly Ser His Asp Asn
1 5

<210> 406
<211> 6
<212> PRT
<213> Homo sapiens

<400> 406
His Gly Ser His Asp Asn
1 5

- 87 -

<210> 407
<211> 6
<212> PRT
<213> Homo sapiens

<400> 407
Lys Gly Ser His Asp Asn
1 5

<210> 408
<211> 6
<212> PRT
<213> Homo sapiens

<400> 408
Gln Gly Ser His Asp Asn
1 5

<210> 409
<211> 6
<212> PRT
<213> Homo sapiens

<400> 409
Thr Gly Ser His Asp Asn
1 5

<210> 410
<211> 6
<212> PRT
<213> Homo sapiens

<400> 410
Ala Gly Ser His Asp Asn
1 5

<210> 411
<211> 6
<212> PRT
<213> Homo sapiens

<400> 411
Leu Gly Ser His Asp Asn
1 5

<210> 412
<211> 6
<212> PRT
<213> Homo sapiens

<400> 412
Pro Gly Ser His Asp Asn
1 5

<210> 413
<211> 6
<212> PRT
<213> Homo sapiens

- 88 -

<400> 413
Phe Gly Ser His Asp Asn
1 5

<210> 414
<211> 6
<212> PRT
<213> Homo sapiens

<400> 414
His Asp Ser His Asp Asn
1 5

<210> 415
<211> 6
<212> PRT
<213> Homo sapiens

<400> 415
His Cys Ser His Asp Asn
1 5

<210> 416
<211> 6
<212> PRT
<213> Homo sapiens

<400> 416
His His Ser His Asp Asn
1 5

<210> 417
<211> 6
<212> PRT
<213> Homo sapiens

<400> 417
His Arg Ser His Asp Asn
1 5

<210> 418
<211> 6
<212> PRT
<213> Homo sapiens

<400> 418
His Thr Ser His Asp Asn
1 5

<210> 419
<211> 6
<212> PRT
<213> Homo sapiens

<400> 419
His Gly Ser His Asp Asn
1 5

- 89 -

<210> 420
<211> 6
<212> PRT
<213> Homo sapiens

<400> 420
His Val Ser His Asp Asn
1 5

<210> 421
<211> 6
<212> PRT
<213> Homo sapiens

<400> 421
His Met Ser His Asp Asn
1 5

<210> 422
<211> 6
<212> PRT
<213> Homo sapiens

<400> 422
His Leu Ser His Asp Asn
1 5

<210> 423
<211> 6
<212> PRT
<213> Homo sapiens

<400> 423
His Ile Ser His Asp Asn
1 5

<210> 424
<211> 6
<212> PRT
<213> Homo sapiens

<400> 424
His Pro Ser His Asp Asn
1 5

<210> 425
<211> 6
<212> PRT
<213> Homo sapiens

<400> 425
His Trp Ser His Asp Asn
1 5

<210> 426
<211> 6
<212> PRT
<213> Homo sapiens

<400> 426

- 90 -

His Gly Asp His Asp Asn
1 5

<210> 427
<211> 6
<212> PRT
<213> Homo sapiens

<400> 427
His Gly Ser His Asp Asn
1 5

<210> 428
<211> 6
<212> PRT
<213> Homo sapiens

<400> 428
His Gly Tyr His Asp Asn
1 5

<210> 429
<211> 6
<212> PRT
<213> Homo sapiens

<400> 429
His Gly His His Asp Asn
1 5

<210> 430
<211> 6
<212> PRT
<213> Homo sapiens

<400> 430
His Gly Arg His Asp Asn
1 5

<210> 431
<211> 6
<212> PRT
<213> Homo sapiens

<400> 431
His Gly Asn His Asp Asn
1 5

<210> 432
<211> 6
<212> PRT
<213> Homo sapiens

<400> 432
His Gly Thr His Asp Asn
1 5

<210> 433
<211> 6

- 91 -

<212> PRT
<213> Homo sapiens

<400> 433
His Gly Gly His Asp Asn
1 5

<210> 434
<211> 6
<212> PRT
<213> Homo sapiens

<400> 434
His Gly Ala His Asp Asn
1 5

<210> 435
<211> 6
<212> PRT
<213> Homo sapiens

<400> 435
His Gly Ile His Asp Asn
1 5

<210> 436
<211> 6
<212> PRT
<213> Homo sapiens

<400> 436
His Gly Pro His Asp Asn
1 5

<210> 437
<211> 6
<212> PRT
<213> Homo sapiens

<400> 437
His Gly Trp His Asp Asn
1 5

<210> 438
<211> 6
<212> PRT
<213> Homo sapiens

<400> 438
His Gly Phe His Asp Asn
1 5

<210> 439
<211> 6
<212> PRT
<213> Homo sapiens

<400> 439
His Gly Ser His Asp Asn
1 5

- 92 -

<210> 440
<211> 6
<212> PRT
<213> Homo sapiens

<400> 440
His Gly Ser Arg Asp Asn
1 5

<210> 441
<211> 6
<212> PRT
<213> Homo sapiens

<400> 441
His Gly Ser Thr Asp Asn
1 5

<210> 442
<211> 6
<212> PRT
<213> Homo sapiens

<400> 442
His Gly Ser Ala Asp Asn
1 5

<210> 443
<211> 6
<212> PRT
<213> Homo sapiens

<400> 443
His Gly Ser Val Asp Asn
1 5

<210> 444
<211> 6
<212> PRT
<213> Homo sapiens

<400> 444
His Gly Ser Leu Asp Asn
1 5

<210> 445
<211> 6
<212> PRT
<213> Homo sapiens

<400> 445
His Gly Ser Ile Asp Asn
1 5

<210> 446
<211> 6
<212> PRT
<213> Homo sapiens

- 93 -

<400> 446
His Gly Ser Phe Asp Asn
1 5

<210> 447
<211> 6
<212> PRT
<213> Homo sapiens

<400> 447
His Gly Ser His Asp Asn
1 5

<210> 448
<211> 6
<212> PRT
<213> Homo sapiens

<400> 448
His Gly Ser His Ser Asn
1 5

<210> 449
<211> 6
<212> PRT
<213> Homo sapiens

<400> 449
His Gly Ser His Tyr Asn
1 5

<210> 450
<211> 6
<212> PRT
<213> Homo sapiens

<400> 450
His Gly Ser His His Asn
1 5

<210> 451
<211> 6
<212> PRT
<213> Homo sapiens

<400> 451
His Gly Ser His Arg Asn
1 5

<210> 452
<211> 6
<212> PRT
<213> Homo sapiens

<400> 452
His Gly Ser His Asn Asn
1 5

- 94 -

<210> 453
<211> 6
<212> PRT
<213> Homo sapiens

<400> 453
His Gly Ser His Gly Asn
1 5

<210> 454
<211> 6
<212> PRT
<213> Homo sapiens

<400> 454
His Gly Ser His Ala Asn
1 5

<210> 455
<211> 6
<212> PRT
<213> Homo sapiens

<400> 455
His Gly Ser His Val Asn
1 5

<210> 456
<211> 6
<212> PRT
<213> Homo sapiens

<400> 456
His Gly Ser His Ile Asn
1 5

<210> 457
<211> 6
<212> PRT
<213> Homo sapiens

<400> 457
His Gly Ser His Asp Ser
1 5

<210> 458
<211> 6
<212> PRT
<213> Homo sapiens

<400> 458
His Gly Ser His Asp His
1 5

<210> 459
<211> 6
<212> PRT
<213> Homo sapiens

<400> 459

- 95 -

His Gly Ser His Asp Lys
1 5

<210> 460
<211> 6
<212> PRT
<213> Homo sapiens

<400> 460
His Gly Ser His Asp Arg
1 5

<210> 461
<211> 6
<212> PRT
<213> Homo sapiens

<400> 461
His Gly Ser His Asp Asn
1 5

<210> 462
<211> 6
<212> PRT
<213> Homo sapiens

<400> 462
His Gly Ser His Asp Thr
1 5

<210> 463
<211> 6
<212> PRT
<213> Homo sapiens

<400> 463
His Gly Ser His Asp Gly
1 5

<210> 464
<211> 6
<212> PRT
<213> Homo sapiens

<400> 464
His Gly Ser His Asp Ala
1 5

<210> 465
<211> 6
<212> PRT
<213> Homo sapiens

<400> 465
His Gly Ser His Asp Leu
1 5

<210> 466
<211> 6

- 96 -

<212> PRT
<213> Homo sapiens

<400> 466
His Gly Ser His Asp Ile
1 5

<210> 467
<211> 6
<212> PRT
<213> Homo sapiens

<400> 467
His Gly Ser His Asp Pro
1 5

<210> 468
<211> 6
<212> PRT
<213> Homo sapiens

<400> 468
His Gly Ser His Asp Trp
1 5

<210> 469
<211> 6
<212> PRT
<213> Homo sapiens

<400> 469
His Gly Ser His Asp Phe
1 5

<210> 470
<211> 13
<212> PRT
<213> Homo sapiens

<400> 470
Ser Gly Gly Arg Ser Asn Ile Gly Asp Asn Thr Val Lys
1 5 10

<210> 471
<211> 13
<212> PRT
<213> Homo sapiens

<400> 471
Ser Gly Gly Arg Ser Asn Ile Gly Cys Asn Thr Val Lys
1 5 10

<210> 472
<211> 13
<212> PRT
<213> Homo sapiens

<400> 472
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Thr Val Lys
1 5 10

- 97 -

<210> 473
<211> 13
<212> PRT
<213> Homo sapiens

<400> 473
Ser Gly Gly Arg Ser Asn Ile Gly Tyr Asn Thr Val Lys
1 5 10

<210> 474
<211> 13
<212> PRT
<213> Homo sapiens

<400> 474
Ser Gly Gly Arg Ser Asn Ile Gly Lys Asn Thr Val Lys
1 5 10

<210> 475
<211> 13
<212> PRT
<213> Homo sapiens

<400> 475
Ser Gly Gly Arg Ser Asn Ile Gly Arg Asn Thr Val Lys
1 5 10

<210> 476
<211> 13
<212> PRT
<213> Homo sapiens

<400> 476
Ser Gly Gly Arg Ser Asn Ile Gly Asn Asn Thr Val Lys
1 5 10

<210> 477
<211> 13
<212> PRT
<213> Homo sapiens

<400> 477
Ser Gly Gly Arg Ser Asn Ile Gly Thr Asn Thr Val Lys
1 5 10

<210> 478
<211> 13
<212> PRT
<213> Homo sapiens

<400> 478
Ser Gly Gly Arg Ser Asn Ile Gly Pro Asn Thr Val Lys
1 5 10

<210> 479
<211> 13
<212> PRT
<213> Homo sapiens

- 98 -

<400> 479
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asp Thr Val Lys
1 5 10

<210> 480
<211> 13
<212> PRT
<213> Homo sapiens

<400> 480
Ser Gly Gly Arg Ser Asn Ile Gly Ser Glu Thr Val Lys
1 5 10

<210> 481
<211> 13
<212> PRT
<213> Homo sapiens

<400> 481
Ser Gly Gly Arg Ser Asn Ile Gly Ser Ser Thr Val Lys
1 5 10

<210> 482
<211> 13
<212> PRT
<213> Homo sapiens

<400> 482
Ser Gly Gly Arg Ser Asn Ile Gly Ser Tyr Thr Val Lys
1 5 10

<210> 483
<211> 13
<212> PRT
<213> Homo sapiens

<400> 483
Ser Gly Gly Arg Ser Asn Ile Gly Ser His Thr Val Lys
1 5 10

<210> 484
<211> 13
<212> PRT
<213> Homo sapiens

<400> 484
Ser Gly Gly Arg Ser Asn Ile Gly Ser Lys Thr Val Lys
1 5 10

<210> 485
<211> 13
<212> PRT
<213> Homo sapiens

<400> 485
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Thr Val Lys
1 5 10

- 99 -

<210> 486
<211> 13
<212> PRT
<213> Homo sapiens

<400> 486
Ser Gly Gly Arg Ser Asn Ile Gly Ser Gln Thr Val Lys
1 5 10

<210> 487
<211> 13
<212> PRT
<213> Homo sapiens

<400> 487
Ser Gly Gly Arg Ser Asn Ile Gly Ser Thr Thr Val Lys
1 5 10

<210> 488
<211> 13
<212> PRT
<213> Homo sapiens

<400> 488
Ser Gly Gly Arg Ser Asn Ile Gly Ser Gly Thr Val Lys
1 5 10

<210> 489
<211> 13
<212> PRT
<213> Homo sapiens

<400> 489
Ser Gly Gly Arg Ser Asn Ile Gly Ser Met Thr Val Lys
1 5 10

<210> 490
<211> 13
<212> PRT
<213> Homo sapiens

<400> 490
Ser Gly Gly Arg Ser Asn Ile Gly Ser Ile Thr Val Lys
1 5 10

<210> 491
<211> 13
<212> PRT
<213> Homo sapiens

<400> 491
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Asp Val Lys
1 5 10

<210> 492
<211> 13
<212> PRT
<213> Homo sapiens

<400> 492

- 100 -

Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Cys Val Lys
1 5 10

<210> 493
<211> 13
<212> PRT
<213> Homo sapiens

<400> 493
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Ser Val Lys
1 5 10

<210> 494
<211> 13
<212> PRT
<213> Homo sapiens

<400> 494
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Tyr Val Lys
1 5 10

<210> 495
<211> 13
<212> PRT
<213> Homo sapiens

<400> 495
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn His Val Lys
1 5 10

<210> 496
<211> 13
<212> PRT
<213> Homo sapiens

<400> 496
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Lys Val Lys
1 5 10

<210> 497
<211> 13
<212> PRT
<213> Homo sapiens

<400> 497
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Arg Val Lys
1 5 10

<210> 498
<211> 13
<212> PRT
<213> Homo sapiens

<400> 498
Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Asn Val Lys
1 5 10

<210> 499
<211> 13

- 101 -

<212> PRT

<213> Homo sapiens

<400> 499

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Gln	Val	Lys
1				5					10			

<210> 500

<211> 13

<212> PRT

<213> Homo sapiens

<400> 500

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Thr	Val	Lys
1				5					10			

<210> 501

<211> 13

<212> PRT

<213> Homo sapiens

<400> 501

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Ala	Val	Lys
1				5					10			

<210> 502

<211> 13

<212> PRT

<213> Homo sapiens

<400> 502

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Val	Val	Lys
1				5					10			

<210> 503

<211> 13

<212> PRT

<213> Homo sapiens

<400> 503

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Leu	Val	Lys
1				5					10			

<210> 504

<211> 13

<212> PRT

<213> Homo sapiens

<400> 504

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Ile	Val	Lys
1				5					10			

<210> 505

<211> 13

<212> PRT

<213> Homo sapiens

<400> 505

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn	Pro	Val	Lys
1				5					10			

- 102 -

<210> 506
<211> 7
<212> PRT
<213> Homo sapiens

<400> 506
Asp Asn Asp Gln Arg Pro Ser
1 5

<210> 507
<211> 7
<212> PRT
<213> Homo sapiens

<400> 507
Glu Asn Asp Gln Arg Pro Ser
1 5

<210> 508
<211> 7
<212> PRT
<213> Homo sapiens

<400> 508
Cys Asn Asp Gln Arg Pro Ser
1 5

<210> 509
<211> 7
<212> PRT
<213> Homo sapiens

<400> 509
Ser Asn Asp Gln Arg Pro Ser
1 5

<210> 510
<211> 7
<212> PRT
<213> Homo sapiens

<400> 510
Tyr Asn Asp Gln Arg Pro Ser
1 5

<210> 511
<211> 7
<212> PRT
<213> Homo sapiens

<400> 511
His Asn Asp Gln Arg Pro Ser
1 5

<210> 512
<211> 7
<212> PRT
<213> Homo sapiens

- 103 -

<400> 512
Lys Asn Asp Gln Arg Pro Ser
1 5

<210> 513
<211> 7
<212> PRT
<213> Homo sapiens

<400> 513
Arg Asn Asp Gln Arg Pro Ser
1 5

<210> 514
<211> 7
<212> PRT
<213> Homo sapiens

<400> 514
Asn Asn Asp Gln Arg Pro Ser
1 5

<210> 515
<211> 7
<212> PRT
<213> Homo sapiens

<400> 515
Gln Asn Asp Gln Arg Pro Ser
1 5

<210> 516
<211> 7
<212> PRT
<213> Homo sapiens

<400> 516
Thr Asn Asp Gln Arg Pro Ser
1 5

<210> 517
<211> 7
<212> PRT
<213> Homo sapiens

<400> 517
Gly Asn Asp Gln Arg Pro Ser
1 5

<210> 518
<211> 7
<212> PRT
<213> Homo sapiens

<400> 518
Ala Asn Asp Gln Arg Pro Ser
1 5

- 104 -

<210> 519
<211> 7
<212> PRT
<213> Homo sapiens

<400> 519
Val Asn Asp Gln Arg Pro Ser
1 5

<210> 520
<211> 7
<212> PRT
<213> Homo sapiens

<400> 520
Met Asn Asp Gln Arg Pro Ser
1 5

<210> 521
<211> 7
<212> PRT
<213> Homo sapiens

<400> 521
Leu Asn Asp Gln Arg Pro Ser
1 5

<210> 522
<211> 7
<212> PRT
<213> Homo sapiens

<400> 522
Ile Asn Asp Gln Arg Pro Ser
1 5

<210> 523
<211> 7
<212> PRT
<213> Homo sapiens

<400> 523
Pro Asn Asp Gln Arg Pro Ser
1 5

<210> 524
<211> 7
<212> PRT
<213> Homo sapiens

<400> 524
Trp Asn Asp Gln Arg Pro Ser
1 5

<210> 525
<211> 7
<212> PRT
<213> Homo sapiens

<400> 525

- 105 -

Phe Asn Asp Gln Arg Pro Ser
1 5

<210> 526
<211> 7
<212> PRT
<213> Homo sapiens

<400> 526
Gly Asn Asp Ser Arg Pro Ser
1 5

<210> 527
<211> 7
<212> PRT
<213> Homo sapiens

<400> 527
Gly Asn Asp Tyr Arg Pro Ser
1 5

<210> 528
<211> 7
<212> PRT
<213> Homo sapiens

<400> 528
Gly Asn Asp Arg Arg Pro Ser
1 5

<210> 529
<211> 7
<212> PRT
<213> Homo sapiens

<400> 529
Gly Asn Asp Gln Arg Pro Ser
1 5

<210> 530
<211> 7
<212> PRT
<213> Homo sapiens

<400> 530
Gly Asn Asp Thr Arg Pro Ser
1 5

<210> 531
<211> 7
<212> PRT
<213> Homo sapiens

<400> 531
Gly Asn Asp Ala Arg Pro Ser
1 5

<210> 532
<211> 7

- 106 -

<212> PRT

<213> Homo sapiens

<400> 532

Gly Asn Asp Ile Arg Pro Ser
1 5

<210> 533

<211> 7

<212> PRT

<213> Homo sapiens

<400> 533

Gly Asn Asp Pro Arg Pro Ser
1 5

<210> 534

<211> 12

<212> PRT

<213> Homo sapiens

<400> 534

Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 535

<211> 12

<212> PRT

<213> Homo sapiens

<400> 535

Gln Ser Tyr Cys Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 536

<211> 12

<212> PRT

<213> Homo sapiens

<400> 536

Gln Ser Tyr Ser Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 537

<211> 12

<212> PRT

<213> Homo sapiens

<400> 537

Gln Ser Tyr Tyr Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 538

<211> 12

<212> PRT

<213> Homo sapiens

<400> 538

Gln Ser Tyr Asn Arg Gly Thr His Pro Ala Leu Leu
1 5 10

- 107 -

<210> 539
<211> 12
<212> PRT
<213> Homo sapiens

<400> 539
Gln Ser Tyr Gln Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 540
<211> 12
<212> PRT
<213> Homo sapiens

<400> 540
Gln Ser Tyr Thr Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 541
<211> 12
<212> PRT
<213> Homo sapiens

<400> 541
Gln Ser Tyr Gly Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 542
<211> 12
<212> PRT
<213> Homo sapiens

<400> 542
Gln Ser Tyr Ala Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 543
<211> 12
<212> PRT
<213> Homo sapiens

<400> 543
Gln Ser Tyr Leu Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 544
<211> 12
<212> PRT
<213> Homo sapiens

<400> 544
Gln Ser Tyr Ile Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 545
<211> 12
<212> PRT
<213> Homo sapiens

- 108 -

<400> 545
Gln Ser Tyr Trp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 546
<211> 12
<212> PRT
<213> Homo sapiens

<400> 546
Gln Ser Tyr Phe Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 547
<211> 12
<212> PRT
<213> Homo sapiens

<400> 547
Gln Ser Tyr Asp Asp Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 548
<211> 12
<212> PRT
<213> Homo sapiens

<400> 548
Gln Ser Tyr Asp Cys Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 549
<211> 12
<212> PRT
<213> Homo sapiens

<400> 549
Gln Ser Tyr Asp Ser Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 550
<211> 12
<212> PRT
<213> Homo sapiens

<400> 550
Gln Ser Tyr Asp Tyr Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 551
<211> 12
<212> PRT
<213> Homo sapiens

<400> 551
Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

- 109 -

<210> 552
<211> 12
<212> PRT
<213> Homo sapiens

<400> 552
Gln Ser Tyr Asp Asn Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 553
<211> 12
<212> PRT
<213> Homo sapiens

<400> 553
Gln Ser Tyr Asp Gln Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 554
<211> 12
<212> PRT
<213> Homo sapiens

<400> 554
Gln Ser Tyr Asp Thr Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 555
<211> 12
<212> PRT
<213> Homo sapiens

<400> 555
Gln Ser Tyr Asp Gly Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 556
<211> 12
<212> PRT
<213> Homo sapiens

<400> 556
Gln Ser Tyr Asp Ala Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 557
<211> 12
<212> PRT
<213> Homo sapiens

<400> 557
Gln Ser Tyr Asp Val Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 558
<211> 12
<212> PRT
<213> Homo sapiens

<400> 558

- 110 -

Gln Ser Tyr Asp Met Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 559
<211> 12
<212> PRT
<213> Homo sapiens

<400> 559
Gln Ser Tyr Asp Leu Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 560
<211> 12
<212> PRT
<213> Homo sapiens

<400> 560
Gln Ser Tyr Asp Ile Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 561
<211> 12
<212> PRT
<213> Homo sapiens

<400> 561
Gln Ser Tyr Asp Pro Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 562
<211> 12
<212> PRT
<213> Homo sapiens

<400> 562
Gln Ser Tyr Asp Trp Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 563
<211> 12
<212> PRT
<213> Homo sapiens

<400> 563
Gln Ser Tyr Asp Arg Asp Thr His Pro Ala Leu Leu
1 5 10

<210> 564
<211> 12
<212> PRT
<213> Homo sapiens

<400> 564
Gln Ser Tyr Asp Arg Cys Thr His Pro Ala Leu Leu
1 5 10

<210> 565
<211> 12

- 111 -

<212> PRT

<213> Homo sapiens

<400> 565

Gln	Ser	Tyr	Asp	Arg	Ser	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 566

<211> 12

<212> PRT

<213> Homo sapiens

<400> 566

Gln	Ser	Tyr	Asp	Arg	Tyr	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 567

<211> 12

<212> PRT

<213> Homo sapiens

<400> 567

Gln	Ser	Tyr	Asp	Arg	His	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 568

<211> 12

<212> PRT

<213> Homo sapiens

<400> 568

Gln	Ser	Tyr	Asp	Arg	Arg	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 569

<211> 12

<212> PRT

<213> Homo sapiens

<400> 569

Gln	Ser	Tyr	Asp	Arg	Asn	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 570

<211> 12

<212> PRT

<213> Homo sapiens

<400> 570

Gln	Ser	Tyr	Asp	Arg	Gln	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

<210> 571

<211> 12

<212> PRT

<213> Homo sapiens

<400> 571

Gln	Ser	Tyr	Asp	Arg	Thr	Thr	His	Pro	Ala	Leu	Leu
1				5					10		

- 112 -

<210> 572
<211> 12
<212> PRT
<213> Homo sapiens

<400> 572
Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 573
<211> 12
<212> PRT
<213> Homo sapiens

<400> 573
Gln Ser Tyr Asp Arg Ala Thr His Pro Ala Leu Leu
1 5 10

<210> 574
<211> 12
<212> PRT
<213> Homo sapiens

<400> 574
Gln Ser Tyr Asp Arg Val Thr His Pro Ala Leu Leu
1 5 10

<210> 575
<211> 12
<212> PRT
<213> Homo sapiens

<400> 575
Gln Ser Tyr Asp Arg Leu Thr His Pro Ala Leu Leu
1 5 10

<210> 576
<211> 12
<212> PRT
<213> Homo sapiens

<400> 576
Gln Ser Tyr Asp Arg Ile Thr His Pro Ala Leu Leu
1 5 10

<210> 577
<211> 12
<212> PRT
<213> Homo sapiens

<400> 577
Gln Ser Tyr Asp Arg Pro Thr His Pro Ala Leu Leu
1 5 10

<210> 578
<211> 12
<212> PRT
<213> Homo sapiens

- 113 -

<400> 578
 Gln Ser Tyr Asp Arg Trp Thr His Pro Ala Leu Leu
 1 5 10

<210> 579
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 579
 Gln Ser Tyr Asp Arg Phe Thr His Pro Ala Leu Leu
 1 5 10

<210> 580
 <211> 48
 <212> DNA
 <213> synthetic construct
 <223> nucleotides at positions 16 to 34 can be
 substituted with any nucleotide such that the
 randomized nucleotides represent 12% of the
 sequence

<400> 580
 tgtcccttgg cccagtagt catagctccc actggtcgta cagtaata 48

<210> 581
 <211> 35
 <212> DNA
 <213> synthetic construct

<400> 581
 gacacctcga tcagcggata acaatttcac acagg 35

<210> 582
 <211> 15
 <212> DNA
 <213> synthetic construct

<400> 582
 tggggccaag ggaca 15

<210> 583
 <211> 45
 <212> DNA
 <213> synthetic construct

<400> 583
 attcgctcta taccgttcta ctttgcgctc tttccagacg ttagt 45

<210> 584
 <211> 18
 <212> DNA
 <213> synthetic construct

<400> 584
 attcgctcta taccgttc 18

<210> 585
 <211> 66
 <212> DNA
 <213> synthetic construct
 <223> nucleotides from position 28 to 42 can be
 substituted with any nucleotide such that the

- 114 -

randomized nucleotides represent 12% of the
sequence

<400> 585
gggtcccagtt ccgaagaccc tcgaacccct caggctgctg tcatatgact ggcagtaata 60
gtcagc 66

<210> 586
<211> 15
<212> DNA
<213> synthetic construct

<400> 586
tggggccaag ggaca 15

<210> 587
<211> 24
<212> DNA
<213> synthetic construct

<400> 587
tgaagagacg gtgaccattg tccc 24

<210> 588
<211> 16
<212> DNA
<213> synthetic construct

<400> 588
gacacctcga tcagcg 16

<210> 589
<211> 48
<212> DNA
<213> synthetic construct

<400> 589
gagtcattct cgacttgctg ccgcacctag gacggtcagc ttggtccc 48

<210> 590
<211> 12
<212> PRT
<213> Homo sapiens

<400> 590
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 591
<211> 12
<212> PRT
<213> Homo sapiens

<220>
<223> Xaa is encoded by a randomized codon of sequence
NNS with N being any nucleotide and S being either
deoxycytosine or deoxyguanine

<400> 591
Xaa Xaa Xaa Xaa Xaa Xaa Phe Thr Gly Ser Met Val
1 5 10

<210> 592
<211> 12

- 115 -

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa is encoded by a randomized codon of sequence
NNS with N being any nucleotide and S being either
deoxycytosine or deoxyguanine

<400> 592

Gln Ser Tyr Xaa Xaa Xaa Xaa Xaa Xaa Ser Met Val
1 5 10

<210> 593

<211> 12

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa is encoded by a randomized codon of sequence
NNS with N being any nucleotide and S being either
deoxycytosine or deoxyguanine

<400> 593

Gln Ser Tyr Asp Arg Gly Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10

<210> 594

<211> 100

<212> PRT

<213> Homo sapiens

<400> 594

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 595

<211> 100

<212> PRT

<213> Homo sapiens

<400> 595

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His

- 116 -

```

                20                25                30
Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
      35                40                45
Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
      50                55                60
Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
      65                70                75                80
Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr
      85                90                95
Tyr Cys Ala Arg
      100

```

<210> 596
 <211> 100
 <212> PRT
 <213> Homo sapiens

```

<400> 596
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1                5                10                15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
      20                25                30
Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
      35                40                45
Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
      50                55                60
Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
      65                70                75                80
Met Tyr Leu Gln Met Ser Asn Leu Lys Thr Glu Asp Leu Ala Val Tyr
      85                90                95
Tyr Cys Ala Arg
      100

```

<210> 597
 <211> 100
 <212> PRT
 <213> Homo sapiens

```

<400> 597
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1                5                10                15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
      20                25                30
Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
      35                40                45
Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
      50                55                60
Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
      65                70                75                80

```

- 117 -

Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr
 85 90 95

Tyr Cys Ala Arg
 100

<210> 598

<211> 98

<212> PRT

<213> Homo sapiens

<400> 598

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 599

<211> 98

<212> PRT

<213> Homo sapiens

<400> 599

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr His Cys
 85 90 95

Ala Arg

<210> 600

<211> 98

- 118 -

<212> PRT

<213> Homo sapiens

<400> 600

Glu Val Gln Leu Val Glu Ser Gly Gly Val Val Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Leu Ile Ser Trp Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95
 Ala Lys

<210> 601

<211> 98

<212> PRT

<213> Homo sapiens

<400> 601

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30
 Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 602

<211> 98

<212> PRT

<213> Homo sapiens

<400> 602

Gln Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

- 119 -

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Ser Ser Tyr Thr Asn Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 603
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 603
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Ser
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Arg Ser Lys Ala Asn Ser Tyr Ala Thr Ala Tyr Ala Ala
 50 55 60
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Arg
 100

<210> 604
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 604
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
 50 55 60
 Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr

- 120 -

85 90 95

Tyr Cys Thr Thr
100

<210> 605
<211> 100
<212> PRT
<213> Homo sapiens

<400> 605
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Glu Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60
Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Thr
100

<210> 606
<211> 100
<212> PRT
<213> Homo sapiens

<400> 606
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60
Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Thr
100

<210> 607
<211> 100
<212> PRT
<213> Homo sapiens

- 121 -

<400> 607

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asn Tyr Ala Ala
 50 55 60
 Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Thr
 100

<210> 608

<211> 100

<212> PRT

<213> Homo sapiens

<400> 608

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
 50 55 60
 Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Thr
 100

<210> 609

<211> 100

<212> PRT

<213> Homo sapiens

<400> 609

Glu Val Gln Leu Val Glu Ser Gly Gly Ala Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

- 122 -

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
 50 55 60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95

Tyr Cys Thr Thr
 100

<210> 610
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 610
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Pro Ala Ser Gly Phe Thr Phe Ser Asn His
 20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Gly Asp Ser Gly Tyr Thr Asn Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn Ser Pro Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Val Lys

<210> 611
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 611
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn His
 20 25 30

Tyr Thr Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ser Ser Gly Asn Ser Gly Tyr Thr Asn Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

- 123 -

Val Lys

<210> 612
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 612
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
 20 25 30
 Asp Met Asn Trp Val His Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Thr Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Val Arg

<210> 613
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 613
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
 20 25 30
 Asp Met Asn Trp Ala Arg Lys Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Val Asp Ser Val
 50 55 60
 Lys Arg Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Lys Asn Arg Arg Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95

Val Arg

<210> 614
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 614

- 124 -

Thr Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Glu Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
 20 25 30
 Asp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Phe Leu Tyr
 65 70 75 80
 Gln Gln Met Asn Ser Leu Arg Pro Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95
 Val Arg

<210> 615
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 615
 Glu Val His Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ala Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Asn Gly Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg

<210> 616
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 616
 Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Ile Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Val Ser Ser Asn
 20 25 30
 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Val Ile Tyr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys

- 125 -

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

```
<210> 617
<211> 97
<212> PRT
<213> Homo sapiens
```

<400> 617
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

```
<210> 618
<211> 97
<212> PRT
<213> Homo sapiens
```

<400> 618
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

- 126 -

<210> 619
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 619
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys

<210> 620
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 620
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Lys

<210> 621
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 621
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

- 127 -

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Val Lys

<210> 622
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 622
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asn Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Gly Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 623
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 623
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Gly Asp Ser Val
 50 55 60

- 128 -

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 624

<211> 98

<212> PRT

<213> Homo sapiens

<400> 624

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Thr Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 625

<211> 98

<212> PRT

<213> Homo sapiens

<400> 625

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

- 129 -

<210> 626
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 626
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 627
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 627
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 628
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 628
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr

- 130 -

[illegible]

```
<210> 629
<211> 98
<212> PRT
<213> Homo sapiens
```

```

<400> 629
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1                    5                      10                      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                      45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                      55                      60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                      70                      75                      80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                      90                      95

Ala Arg

```

```
<210> 630
<211> 98
<212> PRT
<213> Homo sapiens
```

```
<400> 630
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
   1                               5                             10                            15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20                25                      30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35              40                        45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50               55                       60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
  65           70             75            80
```

- 131 -

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 631

<211> 98

<212> PRT

<213> Homo sapiens

<400> 631

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 632

<211> 98

<212> PRT

<213> Homo sapiens

<400> 632

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 633

<211> 98

- 132 -

<212> PRT

<213> Homo sapiens

<400> 633

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Lys

<210> 634

<211> 98

<212> PRT

<213> Homo sapiens

<400> 634

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 635

<211> 98

<212> PRT

<213> Homo sapiens

<400> 635

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

- 133 -

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Ala Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 636
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 636
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 637
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 637
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

- 134 -

85

90

95

Ala Lys

<210> 638

<211> 97

<212> PRT

<213> Homo sapiens

<400> 638

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Gly Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 639

<211> 98

<212> PRT

<213> Homo sapiens

<400> 639

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 640

<211> 98

<212> PRT

<213> Homo sapiens

- 135 -

<400> 640

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Leu Arg Ala Arg Leu Cys Ile Thr Val
 85 90 95

Arg Glu

<210> 641

<211> 98

<212> PRT

<213> Homo sapiens

<400> 641

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 642

<211> 98

<212> PRT

<213> Homo sapiens

<400> 642

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

- 136 -

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 643
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 643
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 644
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 644
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

- 137 -

Ala Arg

<210> 645
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 645
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Arg Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 646
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 646
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 647
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 647

- 138 -

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 648
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 648
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Gly Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 649
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 649
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val

- 139 -

50					55					60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Lys														

```
<210> 650
<211> 98
<212> PRT
<213> Homo sapiens
```

```
<400> 650
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
   1                      5                     10                    15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20                   25                    30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                          40                        45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                      55                             60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
  65                70                   75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
           85                       90                     95
Ala Lys
```

```
<210> 651
<211> 98
<212> PRT
<213> Homo sapiens
```

```
<400> 651
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
   1                               5                             10                            15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20                25
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                          45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                                60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
  65              70                    75            80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
           85                 90               95
Ala Arg
```

- 140 -

<210> 652
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 652
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys

<210> 653
 <211> 95
 <212> PRT
 <213> Homo sapiens

<400> 653
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Arg Lys
 85 90 95

<210> 654
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 654
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

- 141 -

[illegible]

```
<210> 655
<211> 98
<212> PRT
<213> Homo sapiens
```

```

<400> 655
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                               10                      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                      45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Ala
      50                      55                      60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Thr Asn Thr Leu Phe
  65                      70                      75                      80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                      90                      95
Ala Arg

```

```
<210> 656
<211> 98
<212> PRT
<213> Homo sapiens
```

```

<400> 656
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20          25          30
Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35          40          45
Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65          70          75          80

```


- 142 -

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 657
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 657
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 658
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 658
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ser Ile Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 659
 <211> 98
 <212> PRT

- 143 -

<213> Homo sapiens

<400> 659

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 660

<211> 98

<212> PRT

<213> Homo sapiens

<400> 660

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 661

<211> 97

<212> PRT

<213> Homo sapiens

<400> 661

Glu Asp Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Pro Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ser Tyr
 20 25 30
 Val Leu His Trp Val Arg Arg Ala Pro Gly Lys Gly Pro Glu Trp Val

- 144 -

35 40 45
 Ser Ala Ile Gly Thr Gly Gly Asp Thr Tyr Tyr Ala Asp Ser Val Met
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Ser Leu Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Ile Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 662
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 662
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
 35 40 45
 Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 663
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 663
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
 35 40 45
 Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

- 145 -

Ala Arg

<210> 664
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 664
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 665
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 665
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
 35 40 45
 Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Met
 50 55 60
 Lys Gly Gln Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg

<210> 666
 <211> 98
 <212> PRT
 <213> Homo sapiens

- 146 -

<400> 666

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 667

<211> 98

<212> PRT

<213> Homo sapiens

<400> 667

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Thr Thr

<210> 668

<211> 98

<212> PRT

<213> Homo sapiens

<400> 668

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1 5 10 15
 Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
 20 25 30
 Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

- 147 -

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
 65 70 75 80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
 85 90 95

Ser Ala

<210> 669

<211> 98

<212> PRT

<213> Homo sapiens

<400> 669

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asp Met Gly Asn Tyr
 20 25 30

Ala Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Glu Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Trp
 65 70 75 80

Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Leu Ala Trp Asp Thr Ser Pro
 85 90 95

Arg Ala

<210> 670

<211> 98

<212> PRT

<213> Homo sapiens

<400> 670

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Asn Gly

- 148 -

<210> 671
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 671
 Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30
 Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95
 Ser Gly

<210> 672
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 672
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Glu Ala Pro Arg Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
 20 25 30
 Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Tyr Asp Asp Leu Leu Pro Ser Gly Val Ser Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95
 Asn Gly

<210> 673
 <211> 99
 <212> PRT
 <213> Homo sapiens

<400> 673
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln

- 149 -

1					5					10					15				
Arg	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser	Ser	Ser	Asn	Ile	Gly	Ala	Gly				
			20				25						30						
Tyr	Val	Val	His	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu				
		35			40						45								
Leu	Ile	Tyr	Gly	Asn	Ser	Asn	Arg	Pro	Ser	Gly	Val	Pro	Asp	Gln	Phe				
		50			55						60								
Ser	Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Thr	Gly	Leu				
65				70						75					80				
Gln	Ser	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Lys	Ala	Trp	Asp	Asn	Ser				
			85						90					95					
Leu	Asn	Ala																	

```
<210> 674
<211> 99
<212> PRT
<213> Homo sapiens
```

```

<400> 674
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
  1                               10                               15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
      20                               25                               30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
      35                               40                               45
Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
      50                               55                               60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
      65                               70                               75                               80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
      85                               90                               95
Leu Ser Gly

```

```
<210> 675
<211> 98
<212> PRT
<213> Homo sapiens
```

```

<400> 675
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1          5          10          15

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
          20          25          30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
          35          40          45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50          55          60

```


- 150 -

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
85 90 95
Arg Gly

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/07946

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/24 C12N15/13 C12N15/63 C12N5/10 C07K16/00
A61K39/395 G01N33/577 C12P21/08 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, MEDLINE, BIOSIS, WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 659 766 A (SCHERING PLOUGH CORP) 28 June 1995 (1995-06-28) page 2, line 47-54 page 3, line 34 -page 4, line 33 claims ---	
A	WO 94 04679 A (GENENTECH INC) 3 March 1994 (1994-03-03) page 5, line 1 -page 7, line 3 example 3 claims --- -/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 August 2000

Date of mailing of the international search report

23/08/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Covone, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/07946

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1997 CARTER R W ET AL: "Production and characterization of monoclonal antibodies to human interleukin-12." Database accession no. PREV199799787174 XP002144460 abstract & HYBRIDOMA, vol. 16, no. 4, 1997, pages 363-369, ISSN: 0272-457X</p> <p style="text-align: center;">----</p>	
A	<p>PINI A ET AL: "Hierarchical affinity maturation of a phage library derived antibody for the selective removal of cytomegalovirus from plasma" JOURNAL OF IMMUNOLOGICAL METHODS,NL,ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, vol. 206, no. 1-2, 7 August 1997 (1997-08-07), pages 171-182, XP004093130 ISSN: 0022-1759 the whole document</p> <p style="text-align: center;">----</p>	
A	<p>IRVING R A ET AL: "Affinity maturation of recombinant antibodies using E.coli mutator cells" IMMUNOTECHNOLOGY,NL,ELSEVIER SCIENCE PUBLISHERS BV, vol. 2, no. 2, 1 June 1996 (1996-06-01), pages 127-143, XP004052677 ISSN: 1380-2933 abstract figure 1</p> <p style="text-align: center;">----</p>	
A	<p>WO 95 24918 A (GENETICS INST) 21 September 1995 (1995-09-21) page 3, line 15 -page 4, line 14</p> <p style="text-align: center;">-----</p>	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 94,95,142 (completely) are directed to a method of treatment of the human/animal body, and claim 139 (partially) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

In view of the large number and also the wording of the claims presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Moreover support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found for only a very small proportion of the compounds and methods claimed. In the present case, the claims also so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear, concise and supported by the experimental data, namely those parts related to human anti-IL-12 antibodies as disclosed at page 140 line 20 - page 141 line 10 (antibody Y61) and at page 143 line 12-line 32 (antibody J695) and in claims 23-26 and 41-44, including nucleic acid coding for said antibodies, host cell to express said nucleic acid and production of said protein. Thus claims 23-31, 35-45, 47-49, 53, 55-58, 60-62, 64, 66-69, 71-73 have been fully searched. The remaining claims have been partially searched, in the light of the supported subject matter.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/07946

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0659766 A	28-06-1995	AU 1209495 A EP 0788545 A WO 9514780 A US 5959085 A ZA 9409227 A	13-06-1995 13-08-1997 01-06-1995 28-09-1999 22-05-1995
WO 9404679 A	03-03-1994	US 5821337 A AU 675916 B AU 2250992 A EP 0590058 A JP 6508267 T US 6054297 A CA 2103059 A EP 0940468 A WO 9222653 A AU 5083193 A	13-10-1998 27-02-1997 12-01-1993 06-04-1994 22-09-1994 25-04-2000 15-12-1992 08-09-1999 23-12-1992 15-03-1994
WO 9524918 A	21-09-1995	AU 689236 B AU 1974995 A CA 2185565 A EP 0750509 A JP 9510444 T ZA 9500960 A	26-03-1998 03-10-1995 21-09-1995 02-01-1997 21-10-1997 10-10-1995